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SEMINAR PROCEEDINGS

HUMAN HEALTH EFFECTS

OF

ENVIRONMENTAL POLLUTION

October 27 , 1983

RESEARCH ADVISORY COMMITTEE

MINISTRY OF THE ENVIRONMENT

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Ministry of the Environment

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Minister

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SEMINAR PROCEEDINGS

"HUMAN HEALTH EFFECTS OF ENVIRONMENTAL POLLUTION"

OCTOBER 27, 1983

Research Advisory Committee
Ministry of the Environment
Province of Ontario

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The Research Advisory Committee is especially thankful to the authors for their contributions and participation.

The cooperation of the Ministry of Health Laboratory Services Branch as hosts of the seminar is appreciated.

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Introduction

The Research Advisory Committee, sensing the need for an educational seminar which would provide staff with some understanding of toxicology, carcinogenicity and risk assessment methods, undertook the development of this seminar on "Human Health Effects of Environmental Pollution." With the assistance of the Canadian Centre for Toxicology, a panel of speakers has been assembled who can best provide staff with a basic understanding of a very complex subject.

Representatives from the Ministries of Health, Labour, and Natural Resources, the Medical Officers of Health and the major municipalities were invited.

The attached lecture notes form the basis of the presentations made by the respective speakers at the seminar.

Disclaimer

These seminar proceedings have been prepared by the speakers at the request of the Research Advisory Committee. The views expressed are those of the speakers and they do not necessarily reflect the views and policies of the Ontario Ministry of the Environment.

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CHEMICAL TOXICANTS: SOURCES AND STRUCTURES

Introduction

Environmental chemistry is a relatively new field in science which has considerable societal impact. Environmental chemistry is basically the study of the interactions of a specific group of chemicals, namely pollutants or xenobiotics with diverse components of our ecosystem. By definition and derivation, xenobiotics refer to chemicals which are foreign [xenos (Greek)] to nature [bios (Greek)] and have been directly or indirectly introduced into the environment as a consequence of some human (homogenic) activity. Since the field of environmental chemistry or environmental science is concerned with [xenobiotic, pollutant] - [environment] interactions, it is not surprising that this is a highly multidisciplinary research area. Environmental scientists may have received previous academic training in engineering, organic, physical or inorganic chemistry, biology, physics, biochemistry, toxicology or mathematics. It is only by applying the expertise derived from all of these diverse academic disciplines can we begin to understand and control environmental contamination. A useful definition of environmental pollution was proposed as follows:

> Environmental pollution is the unfavorable alteration of our surroundings, wholly or largely as a by-product of man's actions, through direct or indirect effects of changes in energy patterns, radiation levels, chemical and physical constitution and abundances of organisms. These changes may affect man directly, or through his supplies of water and of agricultural and other biological products, his physical objects or possessions, or his opportunities for recreation and appreciation of nature. (Environmental Pollution

Panel, President's Science Advisory Committe, U.S.A.).

The potential adverse effects of chemicals on the environment is a highly complex issue, however, the cartoonist Charles Schulz addresses the related problem of heredity vs environment in the following way.

Differentiating host and environmental interactions. © 1955 United Feature Syndicate, Inc.

The solution of the problem addressed in the cartoon is obvious, however, the determination of adverse impacts by environmental chemicals may often require more subjective assessments.

Primary Sources-of Environmental Toxins: Chemical Industries

The chemical industry is a highly complex operation employing either directly or indirectly millions of people worldwide and making hundreds of thousands of products. From an environmental standpoint one is concerned with

TABLE 1

TOP 50 CHEMICALS: Last year's sharp drop sank output beneath 1977's level

Rank 1982	Rank 1981[a]		Production: Billions of lb 1982	Billions of lb 1981	Common units[b] 1982	Common units[b] 1981	Average annual growth 1981-82	1980-81	1977-82	1972-82
1	1	Sulfuric acid	64.61	81.35	32,304 tt	40,675 tt	−20.6%	−8.1%	−3.4%	0.4%
2	5	Nitrogen	35.07	35.51	484 bcf	490 bcf	−1.2	2.3	7.9	9.6
3	2	Ammonia	30.98	38.08	15,488 tt	19,038 tt	−18.6	0.0	−2.5	0.2
4	4	Oxygen	29.66	35.68	358 bcf	431 bcf	−17.0	0.0	−1.9	0.1
5	3	Lime[c]	28.40	37.78	14,202 tt	18,890 tt	−24.8	6.8	−5.6	−3.0
6	6	Ethylene	24.68	29.42	24,683 mp	29,418 mp	−16.2	2.6	−0.6	1.7
7	8	Sodium hydroxide	18.28	20.83	9,141 tt	10,414 tt	−12.2	−10.4	−3.5	−1.1
8	7	Chlorine	18.27	21.12	9,136 tt	10,560 tt	−13.5	−7.5	−2.9	−0.8
9	9	Phosphoric acid	17.05	19.83	8,523 tt	9,914 tt	−14.0	−9.4	−1.0	2.4
10	12	Sodium carbonate[d]	15.79	16.56	7,896 tt	8,281 tt	−4.7	0.1	−0.5	0.6
11	10	Nitric acid	15.22	18.08	7,608 tt	9,039 tt	−15.7	3.5	−0.9	−0.5
12	11	Ammonium nitrate[e]	14.66	17.58	7,331 tt	8,791 tt	−16.6	−3.7	0.4	0.7
13	14	Propylene	12.29	13.48	12,290 mp	13,482 mp	−8.8	−1.4	−1.6	3.8
14	13	Urea[f]	11.84	16.21	5,918 tt	8,103 tt	−27.0	3.5	5.9	5.6
15	15	Ethylene dichloride	9.99	9.97	9,985 mp	9,974 mp	0.2	−10.2	−1.9	2.5
16	16	Benzene	7.90	9.63	1,073 mg	1,308 mg	−18.0	−35.0	−6.9	−1.5
17	24	Toluene[g]	7.67	6.18	1,057 mg	852 mg	24.1	−16.2	−0.2	1.4
18	18	Carbon dioxide[h]	7.38	7.96	3,688 tt	3,982 tt	−7.4	7.0	5.5	8.6
19	17	Methanol	7.26	8.58	7,265 mp	8,577 mp	−15.5	19.9	2.3	1.8
20	19	Ethylbenzene	6.67	7.81	6,674 mp	7,812 mp	−14.6	2.2	−4.3	1.6
21	20	Vinyl chloride	6.50	6.87	6,495 mp	6,874 mp	−5.4	6.3	1.6	2.5
22	21	Styrene	5.93	6.68	5,928 mp	6,680 mp	−11.3	−2.6	−2.9	−0.1
23	22	Xylene	5.29	6.35	735 mg	882 mg	−16.7	−3.0	−2.6	−0.1
24	26	Ethylene oxide	5.00	5.11	5,000 mp	5,109 mp	−2.1	−2.1	2.7	2.4
25	23	Terephthalic acid[i]	4.97	6.23	4,974 mp	6,234 mp	−20.2	3.0	−1.7	5.3
26	27	Hydrochloric acid	4.97	5.00	2,483 tt	2,501 tt	−0.7	−13.6	−1.8	0.6
27	25	Formaldehyde[j]	4.69	5.72	4,691 mp	5,721 mp	−18.2	3.0	−5.0	−1.9
28	30	Ethylene glycol	4.30	4.14	4,295 mp	4,142 mp	3.7	−5.6	3.2	1.3
29	29	Ammonium sulfate	3.57	4.22	1,785 tt	2,111 tt	−15.4	−1.2	−3.9	−0.4
30	28	*p*-Xylene	2.93	4.53	2,926 mp	4,532 mp	−35.4	6.9	−1.6	2.8
31	32	Acetic acid	2.75	3.07	2,751 mp	3,074 mp	−10.4	3.3	1.4	2.1
32	31	Cumene	2.68	3.31	2,678 mp	3,309 mp	−18.7	−4.3	0.3	1.6
33	35	Aluminum sulfate	2.37	2.59	1,186 tt	1,294 tt	−8.3	0.6	−5.5	0.0
34	34	Carbon black	2.29	2.73	2,290 mp	2,730 mp	−16.1	7.2	−7.2	−3.2
35	36	Phenol[k]	2.14	2.58	2,136 mp	2,578 mp	−17.4	0.4	−1.8	1.1
36	39	Acrylonitrile	2.04	2.00	2,041 mp	1,996 mp	2.3	9.1	4.4	6.2
37	40	Vinyl acetate	1.88	1.94	1,876 mp	1,936 mp	−3.1	0.7	3.4	4.5
38	33	Butadiene[l]	1.83	2.99	1,825 mp	2,986 mp	−38.8	6.7	−10.9	−6.4
39	37	Sodium sulfate[m]	1.79	2.33	893 tt	1,164 tt	−23.2	2.2	−5.7	−3.9
40	38	Acetone	1.76	2.14	1,757 mp	2,144 mp	−18.0	3.8	−4.6	0.1
41	41	Calcium chloride[n]	1.75	1.83	875 tt	917 tt	−4.4	13.1	−2.0	−2.9
42	43	Propylene oxide	1.48	1.73	1,477 mp	1,734 mp	−14.8	−1.9	−4.6	−0.3
43	44	Isopropyl alcohol	1.31	1.67	1,310 mp	1,669 mp	−22.2	−9.1	−7.2	−3.1
44	46	Sodium silicate	1.30	1.48	650 tt	738 tt	−12.2	−6.0	−3.1	−0.2
45	45	Titanium dioxide	1.27	1.52	635 tt	761 tt	−16.6	−3.3	−1.6	−0.9
46	42	Cyclohexane	1.27	1.82	1,272 mp	1,819 mp	−30.2	−7.4	−10.7	−5.7
47	47	Sodium tripolyphosphate	1.26	1.39	632 tt	696 tt	−9.2	−11.8	−2.5	−4.8
48	49	Adipic acid	1.20	1.21	1,197 mp	1,210 mp	−1.1	0.8	−4.9	−2.1
49	48	Acetic anhydride	1.06	1.25	1,061 mp	1,248 mp	−15.0	−15.0	−6.7	−3.9
50	50	Ethanol	1.02	1.32	1,022 mp	1,317 mp	−22.7	−9.2	−5.3	−5.8
		TOTAL ORGANICS IN TOP 50	**150.33**	**173.94**			**−13.6%**	**−1.2%**	**−1.3%**	**1.4%**
		TOTAL INORGANICS IN TOP 50	**315.94**	**373.45**			**−15.4%**	**−2.7%**	**−1.3%**	**0.5%**
		GRAND TOTAL OF TOP 50	**466.22**	**547.39**			**−14.8%**	**−2.2%**	**−1.3%**	**0.8%**

a Revised. b tt = thousands of tons; bcf = billions of cubic feet; mp = millions of pounds; mg = millions of gallons. c Except refractory dolomite. d Synthetic and natural. e Original solution. f 100% basis. g All grades. h All forms. i Includes both acid and ester without double counting. j 37% by weight. k Synthetic only. l Rubber grade. m High and low purity. n Solid and liquid.
Sources: Bureau of the Census, Bureau of Mines, International Trade Commission, C&EN estimates

TABLE 2

A. PRODUCTION: Dropped last year in all sections of chemical industry

Industrial production indexes, 1967 = 100	1982	1981	1980	1979	1978	1977	1976	1975	1974	1973	1972	Annual change 1981–82	Annual change 1972–82
Total index	138.6	151.0	147.0	152.5	146.1	138.2	130.5	117.8	129.3	129.8	119.7	–8%	1%
Manufacturing, total	137.6	150.4	146.7	153.6	146.8	138.4	130.3	116.3	129.4	129.8	118.9	–9	1
Nondurable manufacturing	156.2	164.8	161.2	164.0	156.9	150.5	141.8	126.4	134.6	133.8	126.5	–5	2
Chemicals and products	196.1	215.6	207.1	211.8	197.4	185.7	170.9	147.2	159.4	154.5	143.6	–9	3
Chemicals and synthetic materials	196.7	228.6	222.2	234.2	216.7	201.1	185.1	154.1	176.7	171.9	156.4	–14	2
Basic chemicals	157.1	186.8	187.4	192.0	181.9	171.8	161.8	135.9	153.3	147.7	137.7	–16	1
Alkalies and chlorine	99.2	114.7	121.4	132.3	119.7	119.2	119.0	106.5	125.6	123.8	120.2	–14	–2
Gases, coal tar, inorganic pigments	160.0	181.5	184.3	197.7	186.0	174.3	164.4	133.3	165.0	157.8	146.5	–12	1
Basic organic chemicals	192.9	236.7	231.6	233.3	221.2	204.7	189.8	155.3	177.9	170.0	155.3	–19	2
Inorganic chemicals, nec	109.9	125.5	132.4	134.9	129.3	128.5	124.3	112.9	112.6	110.8	107.8	–12	0
Acids and fertilizer materials	116.0	137.2	145.9	142.0	133.6	124.2	117.6	108.4	116.6	113.1	111.5	–15	0
Synthetic materials	277.4	313.7	293.3	320.1	287.6	260.8	232.5	191.0	224.4	221.1	194.7	–12	4
Plastics materials	403.6	429.3	386.5	420.1	366.5	320.3	280.1	213.1	258.5	243.3	217.4	–6	6
Synthetic rubber	84.9	103.3	103.2	130.5	127.5	124.5	118.7	101.2	129.7	135.6	126.9	–18	–4
Man-made fibers	204.2	254.4	250.3	270.8	250.9	236.8	214.3	191.2	214.4	220.1	189.1	–20	1
Chemical products	195.8	203.0	192.4	190.2	179.0	170.9	157.2	140.7	142.8	137.7	131.3	–4	4
Drugs and medicines	266.8	267.1	245.7	238.2	220.6	213.9	191.5	174.1	179.6	170.1	155.5	0	6
Soap and toiletries	171.5	177.0	170.8	171.6	163.8	155.0	143.0	127.7	128.1	129.0	130.3	–3	3
Paints	115.3	127.4	130.5	137.8	132.6	125.8	122.8	114.2	119.2	114.7	118.0	–9	0
Agricultural chemicals	208.7	241.4	230.5	221.6	211.8	201.1	185.4	163.9	144.6	127.0	108.9	–14	7
Petroleum products	121.8	129.7	132.9	143.9	145.2	142.6	133.5	124.1	124.7	128.5	122.1	–6	0
Rubber and plastics products	254.7	274.0	255.7	272.2	253.6	232.3	199.7	166.7	195.2	184.1	172.4	–7	4

nec = not elsewhere classified. **Source:** Federal Reserve Board

B. MINERALS: Fertilizer raw material production fell again in 1982

Thousands of tons	1982	1981	1980	1979	1978	1977	1976	1975	1974	1973	1972	Annual change 1981–82	Annual change 1972–82
Bromine, sold or used[a]	200[b]	189	189	249	223	217	230	204	216	209	193	6%	0%
Calcium chloride, production[c,d]	875	917	811	981	1,031	968	897	828	1,101	858	914[e]	–5	0
Iodine, consumption[f,g]	4.0[b]	4.4	4.4	4.4	4.0	4.3	3.6	3.1	3.8	3.5	3.4	–10	2
Imports[g]	2.8[b]	3.0	3.1	3.1	3.4	3.5	3.2	2.7	4.0	3.1	3.1	–7	–1
Lime, sold or used[h]	14,202[b]	18,890	19,037	20,983	20,484	19,987	20,257	19,159	21,645	21,132	20,332	–25	–4
Lithium, consumption[f,i]	2.9[b]	3.2	3.0	3.2	3.4	4.1	2.8	2.9	3.8	3.2	2.7	–10	1
Phosphate rock													
Marketable production	41,241	59,110	59,982	56,891	55,156	52,091	49,231	48,806	45,676	42,128	40,823	–30	0
Exports	10,849	11,458	15,737	15,827	14,187	14,584	10,398	12,270	13,895	13,873	14,272	–5	–3
Potash (K_2O equivalent)[j]													
Production	1,929[b]	2,377	2,468	2,453	2,484	2,457	2,400	2,501	2,552	2,604	2,659	–17	–3
Imports[k]	5,291[b]	5,287	5,481	5,693	5,189	5,076	4,594	3,797	4,325	3,587	2,961	0	6
Apparent consumption	6,614[b]	6,849	6,999	7,626	6,840	6,605	6,149	5,113	6,086	5,563	4,816	–3	3
Salt, total sold or used[l]	38,700[b]	38,915	40,396	45,820	42,896	43,439	44,218	41,057	46,565	43,939	45,051	–1	–2
Evaporated	5,418[b]	6,209	6,358	6,248	5,872	5,704	5,634	5,372	5,951	5,913	5,879	–13	–1
Rock	13,158[b]	11,871	11,806	14,891	14,688	14,958	15,668	14,283	14,835	12,347	14,434	11	–1
Salt in brine[m]	20,124[b]	20,835	22,231	24,681	22,336	22,777	22,917	21,401	25,779	25,680	24,737	–3	–2
Sodium carbonate, production[d]	8,000[b]	8,281	8,275	8,253	8,290	8,040	7,560	7,130	7,566	7,535	7,523	–3	1
Sulfur, production[n]	10,788	13,388	13,080	13,339	12,318	11,824	11,992	12,610	12,789	12,231	11,444	–19	–1
Apparent consumption[o]	11,088	14,093	15,056	15,145	13,889	12,850	12,060	11,875	12,115	11,463	11,036	–21	0
Frasch, production	4,641	6,997	7,044	7,007	6,226	6,520	7,016	8,077	8,849	8,518	8,165	–34	–6
Recovered elemental, production	4,855	4,695	4,490	4,486	4,478	3,995	3,514	3,326	2,948	2,706	2,184	3	8
Other, production	1,293	1,695	1,547	1,845	1,615	1,310	1,462	1,208	992	1,008	1,096	–24	2

a Elemental bromine sold as such or used in preparation of bromine compounds by primary producers. b Preliminary figure. c 75% $CaCl_2$ equivalent. d Natural and synthetic. e C&EN estimate. f Estimated apparent consumption. g Crude elemental iodine. h Includes Puerto Rican production. i Lithium content basis. j Includes muriate and sulfate of potash, potassium magnesium sulfate, and parent salts; excludes other chemical compounds containing potassium. k Includes nitrate of potash. l Total obtained by all recovery methods that was sold or used by domestic producers (figures differ slightly from U.S. production figures); figures except for 1982 include Puerto Rican production. m Sold or used as such. n Sulfur content basis. o Calculated from shipments plus imports minus exports (sulfur content basis). **Source:** Bureau of Mines

C. ORGANIC CHEMICALS: Steep declines marked output of most products in 1982

Millions of lb unless otherwise noted	Production											Annual change	
	1982[a]	1981	1980	1979	1978	1977	1976	1975	1974	1973	1972	1981–82	1972–82
Acetic acid, synthetic	2,751	2,705	2,977	3,265	2,776	2,570	2,463	2,197	2,584	2,429	2,235	2%	2%
Acetone	1,757	2,144	2,076	2,653	2,519	2,219	1,869	1,640	1,980	1,989	1,818	−18	0
Acrylonitrile	2,041	1,997	1,830	2,018	1,752	1,646	1,518	1,215	1,412	1,354	1,115	2	6
Aniline	557	634	659	690	606	584	544	407	551	458	410	−12	3
Benzene, mg[b,c]	1,073	1,339	1,585	1,673	1,488	1,457	1,425	1,024	1,488	1,453	1,252	−20	−2
Bisphenol A	479	555	530	576	471	455	449	293	370	320	255	−14	6
1,3-Butadiene[d]	1,826	2,986	2,799	3,583	3,515	3,259	3,507	2,597	3,682	3,644	3,527	−39	−6
Butanol	706	809	790	766	756	660	625	490	558	519	590	−13	2
Caprolactam	797	928	905[e]	945	919	867	780	713	668	656	640	−14	2
Carbon disulfide	na	388	377	448	476	505	508	479	782	777	768	na	na
Carbon tetrachloride	588	726	710	714	737	809	857	906	1,163	1,047	997	−19	−5
Cumene	2,678	3,309	3,459	3,917	3,380	2,644	2,716	2,003	2,906	2,665	2,293	−19	2
Cyclohexane	1,272	1,820	1,964	2,425	2,332	2,265	2,187	1,734	2,352	2,123	2,298	−30	−6
Diisodecyl phthalate	108	140	122	175	171	161	143	106	147	171	153	−23	−3
Dodecylbenzene[f]	484	535	563[e]	627	526	526	529	495	533	498	524	−10	−1
Ethanol, synthetic	1,028	1,317	1,451	1,408	1,267	1,339	1,496	1,429	1,618	1,962	1,851	−22	−6
Ethanolamines[g]	403	429	376	420	362	308	286	258	307	293	284	−6	4
Ethyl chloride	339	324	396	582	540	612	669	575	662	660	576	5	−5
Ethylbenzene	6,674	7,813	7,642	8,448	8,385	8,312	5,770	4,822	6,048	5,688	5,676	−15	2
Ethylene	24,683	29,418	28,667	29,904	25,955	25,172	22,475	20,499	23,891	22,329	20,852	−16	2
Ethylene dichloride	9,985	9,974	11,108	11,794	11,001	10,997	8,042	7,977	9,165[h]	9,293[h]	7,809	0	2
Ethylene glycol	4,295	4,143	4,386	4,729	3,904	3,675	3,335	3,809	3,341	3,278	3,761	4	1
Ethylene oxide	5,000	4,937	5,220	5,665	5,012	4,364	4,184	4,467	4,200[i]	4,167	3,962	0	2
2-Ethylhexanol	321	389	367	318	420	493	450	387	395	402	526	−17	−5
Formaldehyde, 37%[j]	4,691	5,721	5,555	5,971	6,381	6,046	5,449	4,558	5,764	6,424	5,652	−20	−2
Isopropyl alcohol	1,310	1,669	1,836	1,900	1,730	1,888	1,936	1,521	1,939	1,835	1,790	−22	−3
Maleic anhydride	259	293	304	323	341	294	264	216	290	282	274	−12	−1
Methanol, synthetic	7,265	8,577	7,153	7,367	6,443	6,453	6,242	5,176	6,878	7,064	6,472	−15	1
Methyl chloride	370	405	362	463	454	476	378	366	493[h]	544[h]	454	−9	−2
Methyl ethyl ketone	462	611	587	656	661	512	428	425	506	541	509	−24	−1
Methyl chloroform	586	614	692	716	644	635	631	459	592	548	441	−5	3
Methylene chloride	524	592	564	633	570	478	538	497	609	520	471	−11	1
Perchloroethylene	585	691	765	773	725	614	669	679	734	706	734	−15	−2
Phenol, synthetic[k]	2,136	2,578	2,568	2,981	2,682	2,338	2,121	1,746	2,298	2,241	2,052	−17	0
Phthalic anhydride	691	870	818	1,013	978	926	902	702	977	1,023	933	−21	−3
Propylene[l]	12,290	13,482	13,676[i]	14,198[i]	13,014[i]	13,328[i]	10,030[i]	8,709[i]	10,475[m]	9,884[m]	8,472[m]	−9	4
Propylene glycol	403	473	488	610	547	489	517	391	510	502	563	−15	−3
Propylene oxide	na	na	1,767	2,249	2,047	1,866	1,823	1,524	1,756	1,753	1,520	na	na
Styrene, monomer	5,928	6,679	6,856	7,484	7,186	6,867	6,301	4,673	5,956	5,975	5,941	−11	0
Terephthalic acid, dimethyl ester[n]	4,974	6,235	6,054	6,159	5,954	5,410	5,051	4,614	4,262	2,564[o]	4,405[p]	−39	1
Toluene, mg[j,l]	1,057	856	1,017	1,010	1,054	1,018	999	705	922	958	916	23	1
Vinyl acetate	1,876	1,936	1,922	1,982	1,692	1,586	1,481	1,290	1,403	1,503	1,211	−3	4
Vinyl chloride	6,495	6,874	6,466	6,389	6,941	5,986	5,677	4,196	5,621	5,351	5,089	−6	2
o-Xylene	799	918	995	1,082	1,013	985	854	703	1,056	1,068	832	−13	0
p-Xylene	2,926	4,532	4,238	4,650	3,516	3,172	2,911	2,484	2,707	2,326	2,208	−35	3

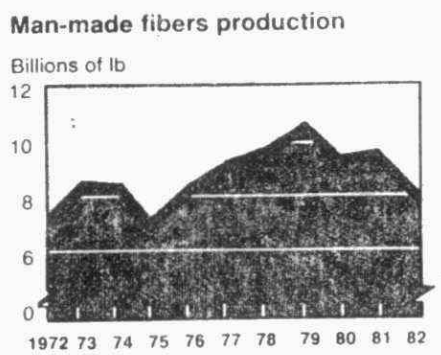
a Preliminary. b Tar distillers not included; includes material for use in blending motor fuels. c All grades. d Rubber grade. e Preliminary figure; final figure not available. f Includes straight-chain hexylbenzene, tridecylbenzene, and other straight-chain alkylbenzenes. g Mono-, di- and triethanolamines. h Production figure may be too low, because some methyl chloride and ethylene dichloride were produced but not separated or measured accurately by some producers. i Approximate. j By weight. k Does not include data for coke ovens and gas retort ovens. l Includes refinery propylene. m Includes data for propane-propylene mixture. n Includes both acid and and dimethyl ester (DMT), without double counting. o Includes only dimethyl ester. p Figure may be too high because of possible double counting of some acid and ester. mg = millions of gallons. na = not available. **Source:** International Trade Commission

D. INORGANICS: Production in 1982 frequently little changed from a decade ago

Thousands of tons unless otherwise noted	Production											Annual Change	
	1982[a]	1981	1980	1979	1978	1977	1976	1975	1974	1973	1972	1981–82	1972–82
Aluminum sulfate[b]	1,186	1,294	1,286	1,315	1,309	1,255	1,202	1,146	1,257	1,233	1,193	−8%	0%
Ammonia,[c,d] 100%	15,488	19,076	19,653	18,634	17,119	17,765	16,716	16,419	15,733	15,208	15,169	−19	0
Ammonium nitrate,[e] 100%	7,331	8,936	9,128	8,293	7,210	7,177	7,186	7,088	7,542	7,235	6,863	−18	1
Ammonium sulfate,[d,f] 100%	1,785	2,194	2,136	2,363	2,449	2,264	2,010	2,106	2,120	1,987	1,858	−19	0
Chlorine gas[g]	9,120	10,767	11,421	12,291	11,052	10,573	10,378	9,167	10,753	10,402	9,854	−15	−1
Hydrochloric acid,[h] 100%	2,483	2,502	2,888	3,090	2,793	2,668	2,542	2,009	2,470	2,534	2,360	−1	1
Hydrogen,[i,j] bcf, 100%	91	103	107	106	90	85	82	74	82	65	59	−12	4
Nitric acid, 100%	7,608	9,077	9,231[k]	8,916[k]	7,934[k]	7,987[k]	7,791[k]	7,527[k]	8,120[k]	8,398[k]	7,981[k]	−16	0
Nitrogen gas,[i,l] bcf, 100%	484	490	479	427	379	328	289	252	243	227	194	−1	10
Oxygen,[i,l] bcf, 100%	358	431	431	456	430	392	388	353	390	389	352	−17	0
Phosphoric acid, 100% P_2O_5	8,523	9,922	10,848	10,318	9,637	8,745	7,949	7,677	7,213	6,927	6,712	−14	2
Phosphorus[m]	361	426	432	460	441	430	437	450	524	526	541	−15	−4
Sodium chlorate, 100%	247	302	312	269	265	251	199	173	203	204	194	−18	2
Sodium hydroxide, 100%	9,141	10,414	11,625	12,759	11,309	11,000	10,516	9,635	11,189	10,734	10,216	−12	−1
Sodium phosphate[n]	632	696	727	757	739	717	724	770	903	967	1,033	−9	−5
Sodium silicate[o]	649	788	805	813	829	760	747	724	770	723	661	−18	0
Sodium sulfate,[p] 100%	902	1,077	1,140	1,121	1,174	1,199	1,232	1,227	1,348	1,437	1,327	−16	−4
Sulfuric acid,[q] 100%	32,304	40,795	44,158	43,204	41,314	38,337	34,878	32,360	33,936	31,949	31,183	−21	−0
Titanium dioxide,[r] 100%	635	761	727	742	701	687	713	603	787	785	693	−17	−1
Urea, 100%	5,931	8,103	7,830	7,000	6,273	5,072	4,498	3,799	3,789	3,543	3,467	−27	6

a Preliminary. **b** Commercial. 17% Al_2O_3; includes municipalities except in 1981. **c** Synthetic anhydrous. **d** Excludes by-product ammonia liquor and ammonium sulfate. **e** Original solution. **f** Synthetic and noncoke by-product. **g** Includes quantities liquefied for use, storage, or shipment. **h** Includes anhydrous hydrochloric acid production. **i** High- and low-purity gas. **j** Liquid and gas; excludes amounts vented and used as fuel, and amounts produced in petroleum refineries for captive use. **k** Includes unspecified amounts of nitric acid produced but not withdrawn from the system. **l** Excludes amounts produced and consumed in making synthetic ammonia or ammonia derivatives. **m** Elemental, white (yellow), and red. **n** Tripolyphosphate (100% $Na_5P_3O_{10}$). **o** Soluble silicate glass (water glass), solid and liquid (anhydrous); excludes amounts produced and consumed in making meta-, ortho-, and sesquisilicates. **p** Anhydrous, high and low purity, and Glauber's salt. **q** Gross (new and fortified). **r** Composite and pure. **Note:** bcf = billions of cubic feet. **Sources:** Department of Commerce, International Trade Commission

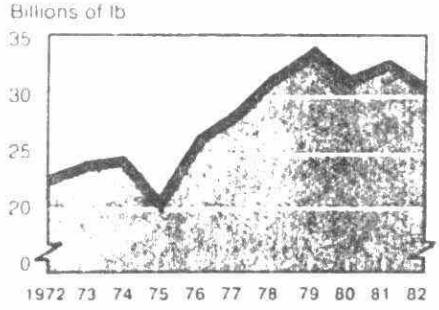
E. MAN-MADE FIBERS: U.S. output in 1982 was the lowest since the mid-1970s



Millions of lb	Production											Annual change	
	1982	1981	1980	1979	1978	1977	1976	1975	1974	1973	1972	1981–82	1972–82
CELLULOSIC FIBERS													
Acetate[a]	196	261	316	324	308	290	298	313	382	462	429	−25%	−8%
Rayon	388	509	490	606	597	598	543	436	817	895	965	−24	−9
NONCELLULOSIC FIBERS													
Acrylic[b]	624	691	779	761	726	709	621	525	631	742	626	−10	0
Nylon[c]	1927	2332	2358	2,720	2550	2326	2075	1857	2124	2175	1974	−17	0
Olefins[d]	723	785	748	759	692	635	577	497	531	492	416	−8	6
Polyester	3169	4176	3989	4,178	3800	3642	3340	2995	2926	2888	2328	−24	3
Textile glass	899	1041	867	1,014	923	787	676	546	683	688	572	−14	5
Other[e]	17	22	19	18	16	16	14	12	12	13	11	−23	4
TOTAL	**7943**	**9817**	**9566**	**10,381**	**9611**	**9002**	**8144**	**7181**	**8105**	**8354**	**7322**	**−20%**	**1%**

a Includes diacetate and triacetate; excludes production for cigarette filtration. **b** Includes modacrylic. **c** Includes aramid. **d** Includes olefin yarn and monofilaments, and olefin and vinyon staple and tow. **e** Includes only yarn and monofilaments; yarn is chiefly saran and spandex. **Source:** Textile Economics Bureau

F. Plastics production



PLASTICS: Output in 1982 dropped back to 1980's depressed level

Millions of lb[a]	Production											Annual change	
	1982	1981	1980	1979	1978	1977	1976	1975	1974	1973	1972	1981-82	1972-82
THERMOSETTING RESINS													
Epoxy[b]	286	336	315	361	299	261	244	198	249	223	184	−15%	4%
Polyester[c]	865	997	947	1,147	1,209	1,061	1,042	834	911	1,051	933	−13	−1
Urea	998	1,165	1,165	1,367	1,122	963	821	690	835	867	739	−14	3
Melamine	158	179	167	200	202	200	186	115	164	170	171	−12	−1
Phenolic[d]	2,006[e]	2,333[e]	1,499	1,781	1,617	1,458	1,340	1,051	1,335	1,387	1,453	−14	na
TOTAL	**4,313**	5,010	**4,094**	**4,857**	**4,448**	**3,943**	**3,631**	**2,888**	**3,494**	**3,698**	**3,481**	**−14**	na
THERMOPLASTIC RESINS													
Polyethylene													
Low density[f]	7,503	7,693	7,291	7,793	7,111	6,471	5,813	4,743	5,973	5,803	5,274	−2	4
High density[g]	4,928	4,695	4,405	5,010	4,201	3,652	3,125	2,468	2,837	2,637	2,325	5	8
Polypropylene	3,477	3,955	3,648	3,841	3,074	2,747	2,632	1,902	2,249	2,162	1,726	−12	7
Acrylonitrile-butadiene-styrene	740	916	920	1,191	1,134	1,069	988	642	857	877	810	−19	−1
Styrene-acrylonitrile	91	107	111	124	120	115	113	109	109	121	99	−15	−1
Polystyrene	3,191	3,621	3,521	4,005	3,823	3,446	3,195	2,662	3,364	3,322	3,139	−12	0
Styrene-butadiene copolymers and other styrene resins	640	829	785	916	878	813	756	585	679	703	624	−23	0
Polyamide, nylon type	228	312	274	327	275	254	247	135	204	205	157	−27	4
Polyvinyl chloride and copolymers	5,326	5,707	5,470	6,119	5,723	5,253	4,716	3,648	4,850	4,562	4,259	−7	2
Other vinyl resins[h]	177	208	196	220	197	197	225	187	209	199	194	−15	−1
TOTAL	**26,301**	**28,043**	**26,621**	**29,546**	**26,536**	**24,017**	**21,870**	**17,081**	**21,331**	**20,591**	**18,607**	**−6%**	**40%**

a Dry weight basis, unless otherwise specified. b Unmodified. c Unsaturated. d Includes other tar acid resins. e Gross weight basis. f 0.940 density and below. g Density above 0.940. h Includes polyvinyl butyral, polyvinyl formal, and polyvinylidene chloride. **na = not available. Source:** Society of the Plastics Industry

SYNTHETIC RUBBER: Output plunged last year from already low levels

Thousands of metric tons	Production											Annual change	
	1982	1981	1980	1979	1978	1977	1976	1975	1974	1973	1972	1981-82	1972-82
Styrene-butadiene rubber[a]	876	1032	1074	1378	1395	1395	1333	1179	1466	1537	1500	−15%	−5%
Polybutadiene	288	342	311	397	378	361	352	290	310	337	299	−16	0
Nitrile	45	66	63	75	73	70	73	55	88	84	75	−32	−5
Ethylene-propylene	123	178	144	175	174	158	130	84	126	120	91	−31	3
Polychloroprene	119	143	151	183	161	165	165	144	163	na	na	−17	na
Butyl				194	154	149	125	80	164	169	131		
Polyisoprene						62	74	61	93	119	134	−31	−5[b]
Other[c]	181[d]	261[d]	272[d]	132[d]	138[d]	56	51	47	88	270[d]	226[d]		
TOTAL	**1632**	**2022**	**2015**	**2534**	**2475**	**2418**	**2304**	**1941**	**2498**	**2627**	**2456**	**−19%**	**−4%**

a Excludes high styrene latex (more than 45% styrene). b Annual change for 1972-82 calculated by comparing polychloroprene plus other (including butyl and polyisoprene) in 1982 with butyl, polyisoprene, and other (including polychloroprene) in 1972. c Excludes polyurethane rubber. d Includes butyl rubber in 1960-82 and polyisoprene in 1978-82. e Includes polychloroprene, isoprene). **na** = not available. **Source:** Rubber Manufacturers Association, Department of Commerce

TABLE 3

TOP 100 CHEMICAL PRODUCERS: In 1982, few eked out an increase in their chemical sales . . .

Rank 1982	Rank 1981[a]	Company	Chemical sales 1982 ($ million)	Change from 1981	Chemical sales as % of total sales	Industry classification	Chemical operating profit[b]	Change from 1981	Chemical operating profits as % of total operating profits	Operating profit margin[c]	Identifiable chemical assets	Chemical assets % of total assets	Operating return on chemical assets[d]
1	1	Du Pont	$10,841	−5.8%	32.5%	Diversified	$417	−63.7%	10.6%	3.8%	$7745	36.6%	5.4%
2	2	Dow Chemical	8,252	−12.1	77.7	Basic chemicals	226	−63.1	63.5	2.7	8124	68.8	2.8
3	3	Exxon	7,259	−14.0	7.1	Petroleum	47	−86.5	0.6	0.6	5047	8.1	0.9
4	4	Monsanto	5,737[e]	−9.1	90.7	Basic chemicals	497	−26.0	99.2	8.7	5234	86.1	9.5
5	5	Union Carbide	4,985	−13.7	55.0	Basic chemicals	294	−49.3	44.7	5.9	6027	57.3	4.9
6	7	Shell Oil	3,085	−14.4	15.4	Petroleum	−56	def	def	def	3841	18.0	def
7	6	Celanese	3,062	−18.4	100.0	Basic chemicals	39	−82.3	100.0	1.3	2862	100.0	1.4
8	8	Standard Oil (Ind.)	2,786	−9.8	9.4	Petroleum	133	−36.7	3.7	4.8	2530	10.6	5.3
9	10	W. R. Grace	2,654	−4.0	43.2	Specialty chemicals	303	−23.5	50.2	11.4	1608	34.5	18.8
10	11	Allied	2,407	−13.7	39.0	Diversified	105	−50.2	13.4	4.4	1726	27.5	6.1
11	12	Phillips Petroleum	2,356	−4.6	15.0	Petroleum	23	−83.6	1.0	1.0	1563	14.9	1.5
12	9	Atlantic Richfield	2,242	−18.1	8.3	Petroleum	−96	def	def	def	2608	12.1	def
13	14	Eastman Kodak	2,151	−8.4	19.9	Photo equipment	205	−29.1	11.0	9.5	2001	19.3	10.2
14	15	Mobil	2,148	−7.6	3.3	Petroleum	6	−95.1	0.1	0.3	2075	5.8	0.3
15	16	Hercules	2,040	−10.3	82.6	Basic chemicals	75	−55.9	65.8	3.7	1468	90.4	5.1
16	13	Gulf Oil	2,006	−18.3	6.6	Petroleum	−329	def	def	def	1232	6.6	def
17	18	Rohm & Haas	1,727	−1.9	94.5	Basic chemicals	156	−16.6	98.7	9.0	1006	90.7	15.5
18	17	American Cyanamid	1,698[e]	−12.7	49.2	Basic chemicals	100	−49.5	43.4	5.9	1308	56.5	7.6
19	19	Stauffer Chemical	1,618	−6.3	100.0	Basic chemicals	285	−7.3	100.0	17.6	1756	100.0	16.2
20	20	American Hoechst	1,506[e]	−6.4	100.0	Basic chemicals	na	na	na	na	na	na	na
21	22	Texaco	1,497	−16.7	3.2	Petroleum	−20	def	def	def	953	4.5	def
22	23	Ethyl	1,411[e]	−6.9	87.4	Basic chemicals	161	4.5	84.5	11.4	959	86.7	16.8
23	28	Air Products[f]	1,359	−0.2	86.6	Basic chemicals	189	−4.2	93.7	13.9	1596	73.6	11.8
24	27	FMC	1,319	−4.6	37.7	Machinery	149	−23.4	81.7	11.3	1084	39.2	13.7
25	31	Ciba-Geigy	1,285	−0.9	68.0	Specialty chemicals	na	na	na	na	na	na	na
26	26	Occidental Petroleum	1,260[g]	−11.5	6.7	Petroleum	15	−86.4	0.8	1.2	1209	8.8	1.1
27	25	Borden	1,220[e]	−14.4	29.7	Dairy products	86	−37.7	29.5	7.0	1271	49.1	6.8
28	30	Ashland Oil	1,197	−10.0	13.1	Petroleum	28	−31.7	8.3	2.3	402	9.5	7.0
29	33	Union Oil of Calif.	1,153	−7.6	10.6	Petroleum	90[h]	−19.3	11.1	7.8	671	7.9	13.4
30	32	Mobay	1,132	−9.4	100.0	Basic chemicals	3[h]	−94.6	100.0	0.3	1213	100.0	0.3
31	21	Tenneco	1,108	−7.5	7.4	Petroleum	15	−53.1	0.8	1.4	963	6.2	1.6
32	24	U.S. Steel	1,077	−24.6	5.7	Steel	−31	def	def	def	592	3.0	def
33	34	Standard Oil of Calif.	1,047	−14.1	3.0	Petroleum	−16[h]	def	def	def	825	4.0	def
34	38	B. F. Goodrich	1,015	−3.1	33.8	Rubber products	1	−96.3	def	0.1	1186	50.0	0.1
35	29	International Minerals[f]	1,013	−17.4	64.9	Agri. chemicals	169	−43.0	79.1	16.7	1345	69.3	12.6
36	35	Olin	1,012	−6.0	55.5	Basic chemicals	22	−53.7	16.6	2.2	914	61.7	2.4
37	35	Diamond Shamrock	996	−12.7	31.3	Petroleum	83	−44.9	34.2	8.3	1024	32.1	8.1
38	37	CF Industries	919[e]	−12.8	100.0	Agri. chemicals	−38	def	def	def	1008	100.0	def
39	41	BASF Wyandotte	895	−7.5	100.0	Basic chemicals	−3	def	def	def	735	100.0	def
40	39	PPG Industries	893	−11.8	30.1	Glass products	39	−67.5	16.4	4.4	1045	32.0	3.7
41	40	Williams Cos.	828[e]	−15.9	49.0	Agri. chemicals	−30	def	def	def	981	51.7	def
42	42	Reichhold Chemicals	815	−14.2	100.0	Basic chemicals	17	−52.8	100.0	2.1	356	100.0	4.8
43	43	Lubrizol	812	−9.7	100.0	Specialty chemicals	103	−34.4	100.0	12.7	569	100.0	18.1
44	44	Eli Lilly	789	−5.8	26.6	Drugs	147	−12.0	20.8	18.6	838	26.6	17.5
45	45	National Distillers	758	−10.9	41.4	Alcoholic beverages	58	−49.1	50.9	7.7	614	35.9	9.4
46	46	Farmland Industries[f]	664	−18.6	11.8	Agri. supplies	−8	def	def	def	486	29.3	def
47	49	Dow Corning	663	−8.2	100.0	Specialty chemicals	53[h]	−30	100.0	8.0	na	na	na
48	51	Nalco Chemical	644	−3.4	100.0	Specialty chemicals	99	−18.9	100.0	15.4	465	100.0	21.3
49	53	Merck	644	−3.0	21.0	Drugs	60	−18.1	9.9	9.3	566	18.6	10.6
50	63	Kaiser Aluminum	642	−0.3	22.1	Nonferrous metals	44	−53.0	80.0	6.9	519	14.2	8.5

a Revised. b Sales less administrative expense and cost of selling. c Operating profit as a percentage of chemical sales. d Operating profit as a percentage of identifiable chemical assets. e Company's sales include significant amounts of nonchemical products. f Fiscal year ended Sept. 30, 1982. g Does not include all chemical products. h Chemical net profit. i Fiscal year

. . . and all who did so ranked in size among the lower 50

Rank 1982	Rank 1981[a]	Company	Chemical sales 1982 ($ millions)	Change from 1981	Chemical sales as % of total sales	Industry classification	Chemical operating profits[b]	Change from 1981	Chemical operating profits as % of total operating profits	Operating profit margin[c]	Identifiable chemical assets	Chemical assets % of total assets	Operating return on chemical assets[d]
51	59	Esmark[k]	$630[e]	−3.0%	18.6%	Food	$ 23	−37.9%	12.6%	3.7%	$370	21.0%	6.2%
52	47	Georgia-Pacific	628	−14.9	11.6	Wood products	75	−49.7	31.9	11.9	1025	24.7	7.3
53	52	Borg-Warner	613	−7.7	19.2	Auto equipment	52	−15.4	22.8	8.5	297	17.7	17.5
54	54	Pennwalt	581	−9.4	61.0	Basic chemicals	46	−36.9	61.7	7.9	438	68.2	10.5
55	68	Standard Oil (Ohio)	554	12.8	4.1	Petroleum	−23	def	def	def	534	3.3	def
56	50	Koppers	535[e]	−21.1	33.8	Diversified	16	−64.1	25.4	2.9	327	29.7	4.7
57	47	Sun Co.	516	−30.8	3.1	Petroleum	na	na	na	na	na	na	na
58	56	Uniroyal	516	−10.0	26.2	Rubber products	42	−46.8	38.1	8.1	425	36.6	9.9
59	64	Witco Chemical	513	0.5	39.3	Specialty chemicals	39	−19.2	34.0	7.5	241	36.4	16.0
60	58	Badische	501	−11.0	100.0	Basic chemicals	−8	def	def	def	483	100.0	def
61	61	Aluminum Co. of America	493[e]	−5.7	10.7	Nonferrous metals	na	na	na	na	na	na	na
62	62	Kerr-McGee	488	−10.0	12.9	Petroleum	−10	def	def	def	656	18.1	def
63	57	NL Industries	477	−15.6	21.5	Petroleum services	61	−21.4	21.4	12.8	307	13.4	19.9
64	65	Airco	473	−5.4	41.7	Basic chemicals	na	na	na	na	na	na	na
65	66	Cabot[l]	471	−4.1	31.0	Specialty metals	62	−4.7	30.1	13.2	264	20.0	23.5
66	55	El Paso	468	−18.8	10.7	Natural gas	−102	def	def	def	331	7.9	def
67	70	National Starch	467	3.8	67.5	Specialty chemicals	na	na	na	na	na	na	na
68	69	International Flavors	448	−0.7	100.0	Specialty chemicals	104	2.0	100.0	23.2	432	100.0	24.1
69	60	Freeport-McMoRan	435	−21.4	58.2	Agricultural chemicals	94	−36.6	130.6	21.6	282	17.0	33.3
70	74	Polysar	432	9.4	100.0	Basic chemicals	na	na	na	na	na	na	na
71	71	Beatrice Foods	419	−6.1	4.6	Food	9	−85.7	1.3	2.1	na	na	na
72	72	Pfizer	391	−7.2	11.3	Drugs	−1	def	def	def	421	11.2	def
73	77	Liquid Air	371	13.4	82.6	Basic chemicals	48	5.1	99.4	12.9	455	84.8	10.5
74	75	Internorth	341	−7.6	8.2	Natural gas	2	−91.1	1.0	0.6	na	na	na
75	—	Henkel	475	nc	100.0	Specialty chemicals	na	na	na	na	na	na	na
76	85	H. B. Fuller[l]	332	0.9	100.0	Specialty chemicals	25	−18.9	100.0	7.5	207	100.0	12.1
77	73	Mississippi Chemical[l]	326	−4.6	100.0	Farm cooperative	25	−67.2	100.0	7.7	375	100.0	6.7
78	78	GAF	311	−4.6	49.8	Specialty chemicals	50	−11.5	210.5	16.1	196	41.9	25.5
79	86	Tyler	302	9.0	34.6	Diversified chemicals	12	−49.8	23.0	3.9	162	35.0	7.4
80	83	Rhône-Poulenc	300	0	100.0	Specialty chemicals	na	na	na	na	na	na	na
81	89	Big Three Industries	283	6.9	36.9	Petroleum services	80	1.1	47.5	28.1	489	46.3	16.4
82	93	Houston Natural Gas[m]	278	12.3	8.7	Natural gas	32	17.5	8.5	11.5	398	17.0	8.0
83	91	Petrolite[k]	275	5.6	90.9	Specialty chemicals	48	9.9	91.0	17.5	161	80.3	29.8
84	80	Terra Chemicals	273	−13.9	100.0	Agricultural chemicals	na	na	na	na	na	na	na
85	79	Upjohn	268	−17.3	14.7	Drugs	−35	def	def	def	183	9.1	def
86	84	Vulcan Materials	263	−12.0	36.5	Nonmetallic minerals	44	−37.2	49.5	16.7	na	na	na
87	88	Emhart	253	−6.2	14.8	Machinery	23	−13.4	12.7	9.1	105	11.5	22.0
88	76	American Petrofina	252	−24.9	11.2	Petroleum	−7	def	def	def	207	19.1	def
89	92	Soltex	250	0	100.0	Basic chemicals	na	na	na	na	na	na	na
90	90	SCM[l]	249[e]	−5.5	13.2	Paint	22	−28.2	26.8	9.0	273	24.3	8.2
91	95	Engelhard	248[e]	3.4	12.3	Specialty metals	49	4.0	57.2	19.8	238	31.5	20.6
92	—	Chemed	233	−1.3	93.6	Specialty chemicals	32	4.3	91.3	13.5	105	63.3	29.9
93	86	Martin Marietta	222	−19.7	6.3	Aerospace	19	−60.1	11.9	8.6	239	9.3	7.9
94	97	Loctite	216	1.2	100.0	Specialty chemicals	26	99.8	100.0	12.0	200	100.0	13.0
95	96	Beker Industries	210	1.6	100.0	Agricultural chemicals	−9	def	def	def	304	100.0	def
96	98	Stepan Chemical	208	1.4	100.0	Specialty chemicals	13	−16.6	100.0	6.0	163	100.0	7.7
97	—	Ferro	205[e]	−13.3	34.5	Specialty materials	8	−42.9	22.7	4.1	114	32.7	7.4
98	99	Getty	202	−1.0	1.6	Petroleum	1	−92.9	0.1	0.5	na	na	na
99	100	Agway	194	−3.0	5.1	Farm cooperative	na	na	na	na	na	na	na
100	—	Great Lakes Chemical	168	12.9	100.0	Specialty chemicals	29	4.7	100.0	17.3	202	100.0	14.4

[a] Ended June 30, 1982. [j] Fiscal year ended Aug. 31, 1982. [k] Fiscal year ended Oct. 31, 1982. [l] Fiscal year ended Nov. 30, 1982. [m] Fiscal year ended July 31, 1982. na = not available. nc = not comparative with previous year. def = deficit.

those chemicals or chemical products which are introduced into the environment and which can perturb the various "spheres" of the environment. It would not be possible to examine all potential chemical pollutants but a number of the broad classes will be noted in this chapter.

All branches of chemistry depend to some extent of basic chemical feedstocks which are used as key intermediates in the production of more specialized chemical products. The data* listed in Table 1 summarize the top 50 chemicals in terms of production figures in the U.S.A. and these are similar to a world top 50 list. Some of these chemicals are themselves pollutants but for the most part they are the crude basic materials for more specialized products; e.g. #3, NH_3 - nitrogen fertilizers; #6, ethylene - polyethylene precursor; #13 propylene - polymers, #21, vinyl chloride - polyvinyl chloride precursor. A breakdown of the chemical production is shown in Table 2A-F and this shows a more comprehensive list of chemicals sold as inorganics, organics (including solvents and polymer precursors), specialty organics, fibres, plastics fertilizers, synthetic rubbers, coatings (i.e. metal oxides etc.). Again these basic chemicals are turned into a broad spectrum of products only a small proportion of which become environmental problems.

The economic impact of the chemical industry is reflected in the sales figure for the top 100 American companies as summarized in Table 3; it is noteworthy that sales of all the top 36 companies exceeds 1 billion dollars per year with the chemical sales of the leader, Dupont Co. of Wilmington, Delaware, exceeding 10.8 billion dollars/year. Table 4 is a comparable industrial list

*All the information shown in Tables 1-5 recently appeared in Chemical and Engineering News which periodically features these summaries to its readers.

which gives the sales of the world's leading chemical or chemically-related industrial companies (excluding those from the USA) and the magnitude of their sales is obvious.

A summary of the Canadian chemical figures is given in Table 5 and most of the top 5 companies are branch plant operations of U.S. multinationals (e.g. Union Carbide, Dupont and Celanese). The data chemical export-import figures reflect a strengthened Canadian export position although both export/import figures were lower in 1982. In the late 1970's all of the Common Market countries with the exception of Italy were net exporters of chemicals to the world and both Canada and Italy were net importers. Italy has maintained this trade imbalance whereas Canada is now a net exporter of chemicals.

The impact of chemistry on the environment is not only reflected by the volume or amount of chemicals produced but by their diversity. Over 3 million chemicals have been described in the chemical literature and several hundred thousand new chemicals are added to this list every year. It has been estimated that only 10,000 chemicals are used and size of this list is increasing at a rate of 500/year. About 300 new compounds enter the environment each year. What about the future of chemical production and its environmental impact? This can probably by illustrated by the following data predictions on the world production of organic chemicals.

- total production (1950) - 7×10^6 tons/year
- total production (1970) - 70×10^6 tons/year
- total production (1985) - 250×10^6 tons/year (estimated)

  20×10^6 tons per year enter the environment.

Although a large portion of the total organics enter the environment each year only a fraction of this material contributes to major pollution problem.

WORLD TRADE: Chemical exports drop

$ Millions	1982	1981	1980	1972
CHEMICAL EXPORTS				
World	$124,500[a]	$139,300[a]	$147,392	$29 137
U.S.	19,891	21,187	20,740	4,134
Canada	3,475	4,002	3,519	639
EC, 10 countries[b]	60,770	69,452	76,710	15,718
Belgium-Luxembourg	5,925	6,477	7,422	1,564
France	9,937	12,141	13,249	2,428
Italy	4,310	5,214	5,505	1,222
Netherlands	9,274	10,418	11,288	2,338
U.K.	8,872	10,831	12,303	2,404
West Germany	19,994	21,966	24,318	5,416
Switzerland	5,440	5,505	5,680	1,525
Japan	6,365	6,841	6,767	1,784
CHEMICAL IMPORTS				
U.S.	9,494	9,446	8,594	2,015
Canada	3,262	3,569	3,249	1,001
EC, 10 countries[b]	48,852	51,483	57,075	11,152
Belgium-Luxembourg	4,972	5,235	5,972	1,213
France	9,889	10,376	12,169	2,185
Italy	6,896	7,020	7,993	1,564
Netherlands	5,378	5,726	6,393	1,356
U.K.	6,427	7,260	7,310	1,630
West Germany	11,824	12,201	13,314	2,509
Switzerland	3,208	3,226	3,648	833
Japan	6,824	6,487	6,202	1,148

a. Estimates. b. European Communities; historical data have been adjusted to reflect present 10 member. **Sources:** United Nations, Organization for Economic Cooperation & Development, European Communities, individual country data

B. U.S. TRADE: Europe takes less, ships more

$ Millions	1982	1981	1980	1972
U.S. CHEMICAL EXPORTS				
Canada	$ 2,183	$ 2,478	$ 2,134	$ 693
Western Europe	5,940	6,155	6,429	1447
Eastern Europe	366	347	88	33
Communist Asia	497	406	386	2
Japan	2,530	2,349	1,964	313
Rest of Asia	2,600	2,597	2,782	348
Middle East	533	517	459	80
Latin America	3,670	4,469	4,846	908
Australasia	655	778	645	128
Africa	524	642	721	108
Other	393	449	286	74
TOTAL	**$19,891**	**$21,187**	**$20,740**	**$4134**
U.S. CHEMICAL IMPORTS				
Canada	$2596	$2916	$2494	$ 439
Western Europe	4174	3931	3953	940
Eastern Europe	151	131	195	15
Communist Asia	136	126	107	2
Japan	879	956	697	250
Rest of Asia	220	197	163	26
Middle East	99	110	58	11
Latin America	613	669	536	82
Australasia	286	186	206	99
Africa	225	117	62	17
Other	115	107	123	134
TOTAL	**$9494**	**$9446**	**$8594**	**$2015**

Source: Department of Commerce

U.S. TRADE BY-PRODUCTS: Exports slump for most categories

$ Millions	Chemical exports[a] 1982	1981	1980	1972[c]	Chemical imports[b] 1982	1981	1980	1972[c]
Organic chemicals	$ 5,440	$ 5,929	$ 5,697	$1134	$2960	$2974	$2541	$ 517
Inorganic chemicals	1,969	2,286	2,106	414	1502	1573	1324	426
Radioactive and associated materials	1,132	988	833	181	1143	786	990	110
Dyeing, tanning, and coloring materials	162	175	186	57	213	226	195	114
Pigments, paints, and varnishes	332	349	304	100	103	102	89	16
Medicinals and pharmaceuticals	2,275	2,165	1,932	474	563	583	509	149
Flavors, perfumes, cosmetics, and toiletries	514	535	479	102	295	289	302	100
Cleaning and polishing materials	324	396	327	93	76	74	72	14
Fertilizers, manufactured	1,386	1,735	2,265	298	963	1181	1105	232
Explosives and pyrotechnics	90	90	87	28	67	61	59	21
Plastics and resins	3,650	3,809	3,884	696	766	780	647	177
Pesticides and disinfectants	607	544	553	d	265	283	264	d
Miscellaneous chemical products	2,010	2,187	2,087	556	579	534	497	140
TOTAL	**$19,891**	**$21,187**	**$20,740**	**$4134**	**$9494**	**$9446**	**$8594**	**$2015**

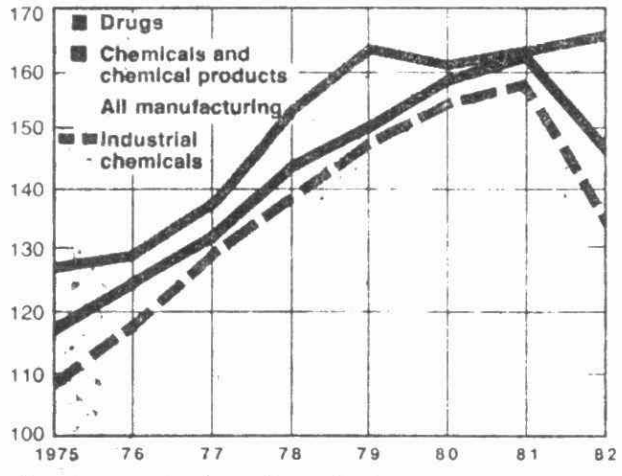
a. Exports of domestic merchandise, F.A.S. (free alongside ship) value. b. General imports, F.A.S. value. c. As a result of statistical reclassification, data prior to 1978 are not comparable to those of subsequent years. C&EN has rearranged earlier data to make them as comparable as possible. d. Included in miscellaneous. **Source:** Department of Commerce

Three strikes and the Canadian chemical industry was out last year.

Strike one was the worst recession that Canada has seen since the Great Depression. Strike two was a downturn in world markets that the Canadian industry depends upon to soak up output from the world-scale plants that it has built in recent years. Strike three was the national energy policies that drove up prices of oil, natural gas, and petrochemical feedstocks when they were declining virtually everywhere else in the world.

The results were predictable. Sales were down. Profits were down. And production cutbacks struck across the board. Fertilizer materials, basic and intermediate petrochemicals, plastics, and inorganics all felt the impact of the domestic and worldwide economic slowdown. Chemical exports dropped almost 12% to $4 billion (Canadian). Several chemical companies chalked up the worst financial results of their history, and at least two companies, Petrosar and Petromont, were on the verge of collapse without government aid.

Production of chemicals nosedives



a Gross domestic product. Source: Statistics Canada

COMPANY RESULTS: Red ink for most firms

$ Millions (Canadian)	Sales	Net income[a]	Profit margin	Capital expenditures	Employees (thousands)
C-I-L INC.[b]					
1982	$1059.8	$15.2	1.4%	$85.5	6.9
1981	1149.9	54.3	4.7	94.1	7.8
1980	1004.2	51.5	5.1	70.2	8.3
1979	880.0	36.3	4.1	84.4	8.0
CELANESE CANADA					
1982	$ 323.9	($5.6)[c]	—	$59.8	3.4
1981	373.0	20.4	5.4%	87.7	3.5
1980	336.2	24.2	7.2	55.1	4.2
1979	353.6	21.2	6.0	24.4	4.4
DU PONT CANADA					
1982	$ 980.0	($7.4)[c]	—	$24.0	5.4
1981	1139.1	33.5	2.9%	47.0	6.1
1980	995.3	42.6	4.3	53.2	5.9
1979	879.6	58.9	6.7	23.3	5.6
POLYSAR					
1982	$1330.0	($30.5)[c]	—	$207.0	6.6
1981	1049.0	74.0	7.1%	58.3	5.4
1980	1171.0	73.4	6.3	86.2	6.1
1979	1136.4	22.0	1.7	199.3	7.1
UNION CARBIDE CANADA					
1982	$ 691.3	($17.9)[c]	—	$ 67.6[d]	4.5
1981	827.3	36.1	4.4%	100.0	5.0
1980	794.8	79.6	10.0	58.9	5.0
1979	685.9	57.8	8.4	30.1	4.8

a Excluding extraordinary gains and losses. b Name changed from Canadian Industries Ltd. on Jan. 1, 1980. c Deficit. d Excludes $50.9 million spent on Prentiss, Alta., ethylene glycol plant that was transferred to another Union Carbide subsidiary.

TRADE BALANCE: Big gain for total surplus

$ Millions (Canadian)	1982	1981	1980	1979
Exports[a]	$81,464	$81,203	$74,446	$64,317
Imports	67,355	79,129	69,274	62,871
Trade balance	14,109	2,074	5,172	1,446

Lower exports hurt chemical surplus

$ Millions (Canadian)	1982	1981	1980	1979
Exports[a]	$4073	$4605	$4056	$3322
Imports	3584	3814	3354	3240
Trade balance	489	791	702	82

a Domestic exports only. Source: Statistics Canada

PRODUCTION: Cutbacks almost everywhere

Thousand of metric tons	1982	1981	1980	1979	Change 1981-82
Ammonia, anhydrous	2509	2654	2555	2405	−5%
Ammonium nitrate	1018	1212	873	914	−16
Ammonium phosphate	888	1355	1466	1336	−34
Benzene	504	571	604	492	
Chlorine	1233	1292	1303	1031	−5
Ethylene	1013	1330	1184	1014	−24
Hydrochloric acid	135	186	177	154	−27
Phosphoric acid, 100% P_2O_5	503	744	808	748	−32
Polyesters, unsaturated	19	26	27	32	−27
Polyethyene	571	633	641	628	−10
Polystyrene	133	123	110	124	8
Polyvinyl chloride	194	222	190	145	−13
Sodium chlorate	264	251	245	224	4
Sodium hydroxide	1367	1458	1459	1138	−6
Sulfuric acid, 100%	3131	4117	4295	3666	−24
Urea	1231	1303	1274	1212	−6

Source: Statistics Canada

Polymeric waste material is disposed of in landfill or garbage dumpsites, many small molecular weight solvents are rapidly biodegraded to harmless non toxic products and this is also the case with many pesticides, nitrogen fertilizers, etc. Thus the effects of chemical pollutants are directly dependent on their structures and their relative stability in the environment. A useful classification of pollutants is shown below.

A) Non Persistent Pollutants

-rapidly biodegradable or photodegradable to harmless non toxic by products.

-these chemicals present problems when used carelessly since their effects are usually manifested immediately on the target organism.

-examples are the organophosphate pesticides.

B) Medium Level Persistent Chemical Pollutants

-these chemicals can have variable environmental half-lives but are sufficiently stable to exert some effects on the environment prior to their decomposition.

-examples are the hydrocarbon oil pollutants and possible some detergents.

C) Persistent Pollutants

-chemicals which are environmentally stable and are only slowly degraded in the environment.

-many of these compounds are lipophilic and as a result are bioaccumulated in higher trophic levels of the food chain and stored in fatty tissue.

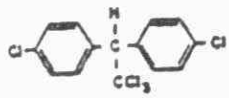
-examples are the polychlorinated biphenyls, DDT, mirex, kepone, dieldrin, aldrin, organomercurials; other halogenated hydrocarbons.

The direct and indirect entry of pollutants into the environment and their subsequent translocation within the ecosystem is illustrated in Figures 1-3.

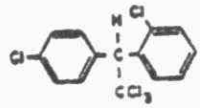
In addition, the following broad classes of chemicals which have become major environmental problems. These include:

1) pesticides
2) food additives
3) nitrogen fertilizers
4) halogenated hydrocarbons
5) oil pollutants
6) solvents
7) detergents
8) industrial compounds (PCB, PBB) and plasticizers
9) combustion induced gases, smokes and hydrocarbons

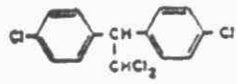
These classes include persistent, medium persistent and biodegradable chemicals which are associated with diverse environmental problems and may contribute to adverse human health effects.



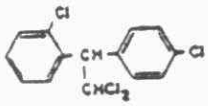
p,p' - DDT



o,p' - DDT



p,p' - DDD



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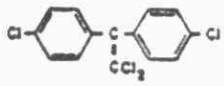
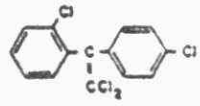
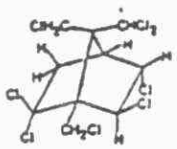
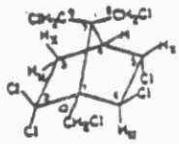


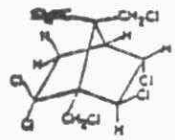
p,p' - DDE



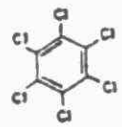
o,p' - DDE



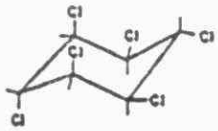




Toxaphene Components



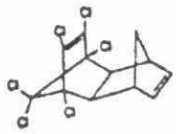
Hexachlorobenzene



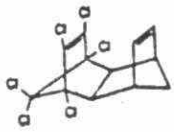
Lindane

Polychlorinated
Biphenyls (PCB)

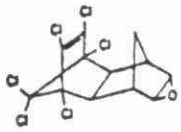
Persistent Chlorinated Pollutants - Pesticides and Industrial Compounds



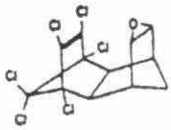
Aldrin



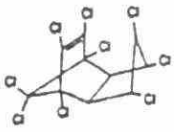
Isodrin



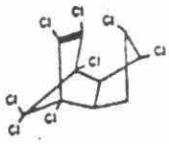
Dieldrin



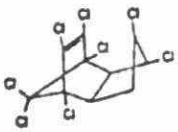
Endrin



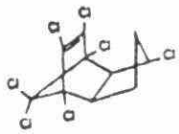
Nonachlor



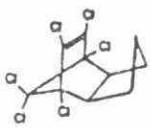
trans-Chlordane



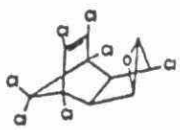
cis-Chlordane



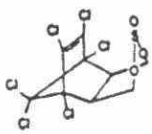
Heptachlor



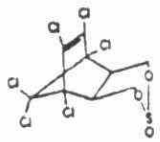
Chlordene



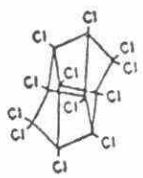
Heptachlor Epoxide



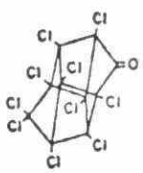
I - Endosulfan



II - Endosulfan



Mirex

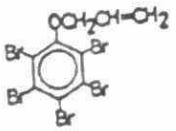


Kepone

Persistent Chlorinated Pollutants - The Hexachlorocyclopentadiene Derived Insecticides

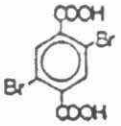
REPRESENTATIVE FLAME RETARDANTS

bromine compounds

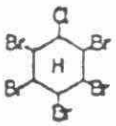


1-(Pentabromophenoxy)-propene(2)

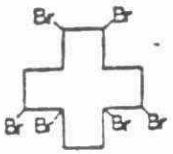
Tetrabromophthalic anhydride



2,5-Dibromoterephthalic acid



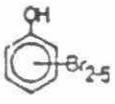
Pentabromochlorocyclohexane



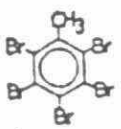
Hexabromocyclododecane

Mono-2,3-dibromopropyl maleate

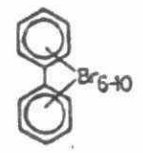
Polybromobenzenes



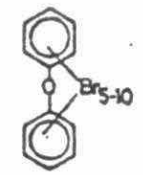
Polybromophenols



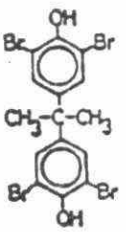
Pentabromotoluene



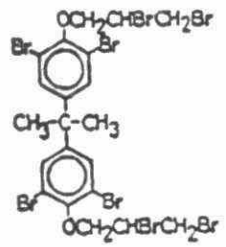
Polybromobiphenyls



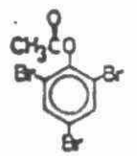
Polybromodiphenyl ethers



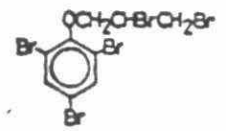
Tetrabromobisphenol A



2,2-Bis-[4-(2,3 dibromopropoxy)-3,5 dibromophenyl]-propane



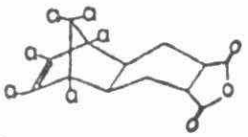
2,4,6-Tribromophenyl acetate



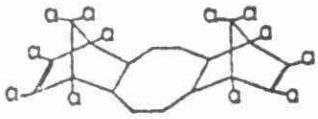
2,4,6-Tribromophenoxy-2,3-dibromo-propane

REPRESENTATIVE FLAME RETARDANTS

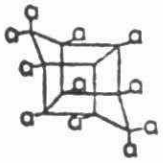
chlorine compounds



Cloran



1,5-Bis(chlorendo)cyclo-octane

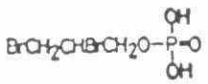


Mirex
(Dechlorane)

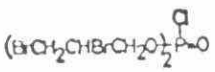
Chlorendic anhydride
(HET anhydride)

$C_nH_{n+2}Cl_n$ Chloroparaffins

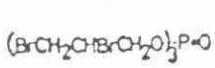
phosphorous compounds



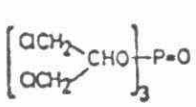
Mono(2,3-dibromopropyl) phosphate



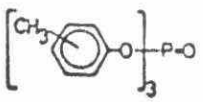
Di(2,3-dibromopropyl) phosphorochloridate



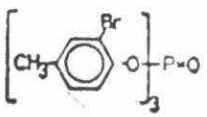
Tris(2,3-dibromopropyl) phosphate



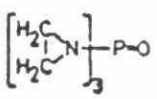
Tris(1,3-dichloroisopropyl) phosphate



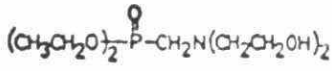
Tricresyl phosphate



Tris(2-bromo-4-methylphenyl) phosphate



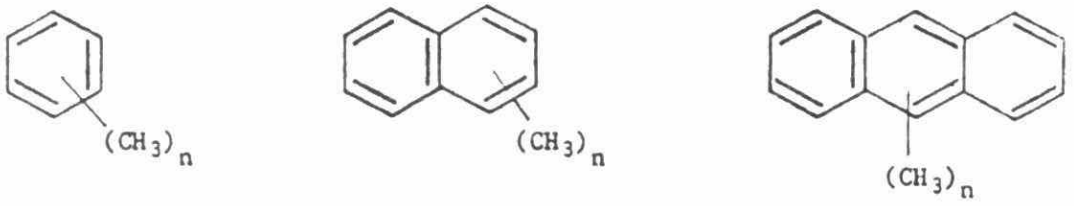
Tris-aziridinyl phosphorous oxide
(APO)



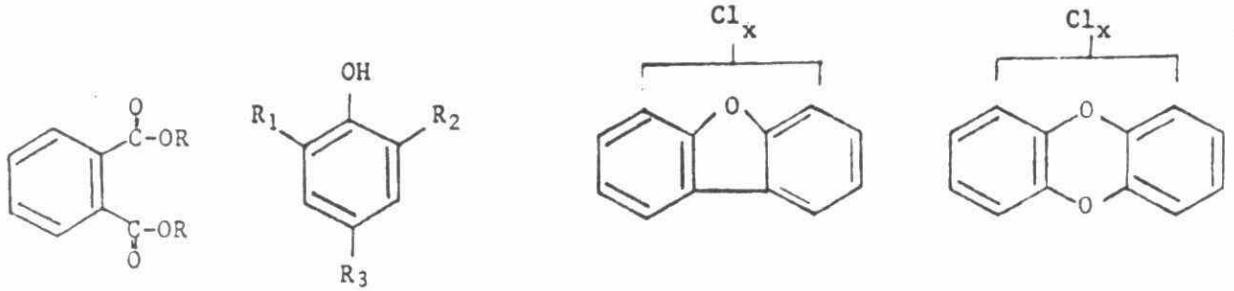
0,0-diethyl-N,N-bis(2-hydroxyethyl)aminomethyl phosphonate

CH_3Cl	CH_2Cl_2	$CHCl_3$	CCl_4
methylchloride	methylene chloride	chloroform	carbon tetrachloride
CH_3Br	CH_2Br_2	$CHBr_3$	CBr_4
methyl bromide	methylene dibromide	bromoform	carbon tetrabromide
CH_2BrCl	$CHBr_2Cl$	$CHBrCl_2$	CCl_2Br_2
bromochloromethane	chlorodibromomethane	bromodichloromethane	dichlorodibromomethane
$\overbrace{(C-C)}^{HxCly}$	$\overbrace{(-C-)_z}^{HxCly}$	$\overbrace{(-C-)_z}^{HxXy}$	$CFCl_3$, CF_2Cl_2
chlorinated ethanes	chlorinated alkanes	halogenated alkanes	freons
$CH_2 = CHCl$	$\overbrace{(CH=CH)}^{Cl_2}$	$CCl_2 = CHCl$	$CCl_2 = CCl_2$
vinyl chloride	dichloroethylenes	trichloroethylene	tetrachloroethylene

Halogenated Hydrocarbon Pollutants - industrial compounds and intermediates, solvents.

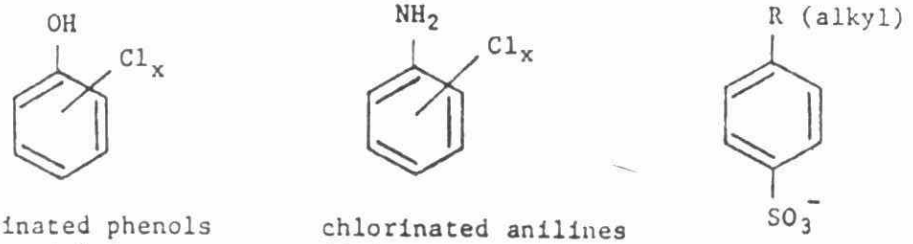


alkylated aromatics - examples of crude oil "water soluble" pollutants



phthalates (plasticizers). phenolics & ethers (e.g. food additives)

chlorinated dibenzofurans and dibenzo-p-dioxins (highly toxic impurities in some industrial products)



chlorinated phenols (industrial intermediates)

chlorinated anilines (industrial intermediates)

(anionic detergents)

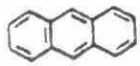
Aromatic Pollutants

Indene

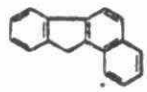
Acenaphthylene

Acenaphthene

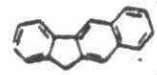
Fluorene



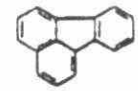
Anthracene



1,2-Benzofluorene

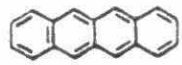


2,3-Benzofluorene

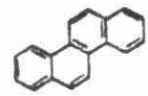


Fluoranthene

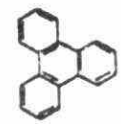
Pyrene



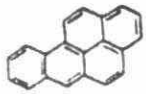
2,3-Benzanthracene



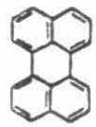
Chrysene



Triphenylene

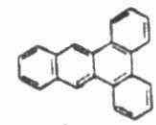


Benzo[a]pyrene*

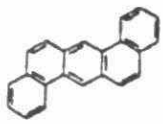


Perylene

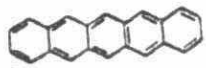
Benzo[ghi]perylene



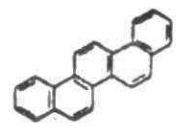
1,2,3,4-Dibenzanthracene*



1,2,5,6-Dibenzanthracene*



Pentacene



Picene

Ovalene

Representative Polynuclear Aromatic Hydrocarbons--pollutants formed on the combustion of organic materials.

* carcinogenic

ENVIRONMENTAL SAMPLING AND ANALYSIS

FOR TOXIC ORGANICS

ENVIRONMENTAL SAMPLING AND ANALYSIS FOR TOXIC ORGANICS

The previous paper provided an overview of the chemistry of organic toxics. We will now examine the requirements for investigating these compounds in the environment, the problems that this investigation can cause for the analyst, and some of the technology used to solve these problems. The analyst may be asked the general question -

"What organic chemicals are present in a specific environmental compartment, and which of them are toxics?"

Is compound A present, at what level is it at present, and what potential for human exposure does its presence have?

Question one is usually a difficult one to answer well. Methodology must capture and detect as wide a range of compounds as possible, and thus is generally a compromise, allowing many compounds to be detected, but not being optimized for accurate measurement of any one. On the other hand, methods for specific toxics can be optimized for precision, accuracy and confidence of identification prior to use. They will generally give more reliable data. It is this area on which I will concentrate in this presentation.

What makes environmental analysis for organic toxics difficult? Most of the problems can be reduced to 4 points:-

1. Sample Complexity
2. Contaminant Concentration Range
3. Variation in Toxicity of Different Contaminants
4. Reactivity of Target Contaminants

1. Sample Complexity

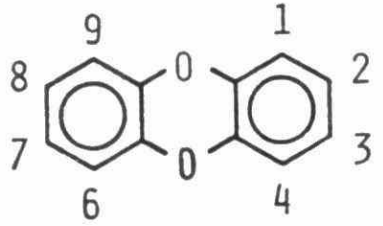
The environment acts as a sink for industrial and natural organics. Currently, there are over a million known organics. An environmental sample extract frequently contains hundreds, if not, thousands of these organic chemicals. These can range in concentration for percentage levels, down to parts per trillion or below. Today's toxicologist has the tools and expertise to detect biological injury such as gene damage, neurotoxic effects or carcinogenicity caused by extremely low levels of toxics. Progress in this field has been accompanied by a general and rapid lowering of the acceptable human exposure levels for toxics. In the 1960s, an acceptable guideline for DDT levels in fish for human consumption was 5 ppm. During the 1970s a Mirex guideline was set at 0.1 ppm and in the 1980s, the extreme toxicity of 2,3,7,8-TCDD has necessitated a guidelines of 20 ppt. The analyst has thus been asked to lower his detection limit from parts per million to parts per trillion, and to specifically isolate, detect and quantitate a single trace organic toxin in the presence of thousands of other compounds, in concentrations up to 10 million times higher than that of the target compound concentration. Many other pollutants have similar chemical and physical behaviour to the target compound and must be quantitatively removed before accurate analysis is possible. For example, Great Lakes fish can contain PCBs at 50 parts per million, chlorinated pesticides of up to 5 ppm, and ppm levels of other chlorinated organics, all of which can interfere with dioxin analysis especially if the dioxins must be determined at the 0-100 ppt level.

2. Toxicity

Specificity in trace organic analysis is also crucial if a given family of organics has a significant range in toxicity. TCDDs - Figure 1 shows the general structure of the dioxin molecule. Addition of 4 chlorine dioxins produces tetrachlorobenzo-p-dioxins, a family of chemically identical compounds with minimal structural differences, but a range in toxicity of up to 10,000 from lowest to highest. (See Table 1). If the analyst fails to separate and properly identify the 22 isomers prior to qualitative, major errors can result in identifying potential health risk.

FIG 1

DIOXIN STRUCTURE



DIBENZO - P - DIOXIN

TABLE 1

COMPARATIVE TOXICITIES OF TCDDS

Compound	LD_{50} (Rat) (mg/kg)
Unsubstituted Dioxin	> 1000
1,2,3,4-TCDD	> 1000
1,3,6,8-TCDD	> 100
2,3,7,8-TCDD	0.04
(2,3,7,8-TBDD)	≃ 1.0
OCDD (8)	≃ 2000

3. Reactivity

Trace analysis for organics is also rendered more difficult by their reactivity and volatility. Sampling and analytical methods for organics must avoid any treatment which can alter the structure of the target compound. The wide range of volatility and chemical activity of organics makes this a difficult task.

SAMPLING AND ANALYSIS PROGRAMS FOR TOXIC ORGANIC POLLUANTS

With the above concerns in mind, what features are necessary for a sampling and analysis program that will provide useful information on the potential for human exposure to a specific organic toxicant? Features of such a program include:

A. Program Design
B. Sampling and Sample Preservation Methods
C. Sample Processing and Analysis
D. Data Quality Assurance
E. Data Interpretation
F. Program Feedback

A. PROGRAM DESIGN

Proper program design requires the following components:

1. Parameter Definition
2. Definition of Enviornmental Interaction and Human Exposure Potential
3. Understanding of Sampling Level Required for Statistically Valid Data
4. Cost

1. Parameter Definition

Precise definition of the parameter to be measured is essential. In the case of dioxin analysis, does the program require analysis for "Total dioxins", "Total TCDDs" or 2,3,7,8-TCDD. Treated drinking water can contain Trihalomethanes, chlorinated compounds (precursors) which can break down during storage and/or analysis to form additional THMs and non chlorinated precursors, which can react with residual chlorine to form chlorinated precursors and thus more THMs. Thus, in a program for the analysis of Trihalomethanes in drinking water, should the result reflect THMs at time of sampling, total of original THMs and THMs

from chlorinated Precursors, or "Terminal THMs", the sum of original THMs, THMs produced from chlorinated precursors, and THMs from the reaction of non chlorinated precursors with chlorine residual? If these definitions are not made prior to program implementation, incorrect sampling and analysis methods and poor quality data will result.

2. Matrix Interaction and Human Exposure Potential

Later in this seminar you will hear how knowledge of the chemical and physical properties of a chemical can be used to predict its probable movement in the environment. This modelling approach allows the program designer to predict in which environmental compartments a toxicant will concentrate. This type of information can indicate if and how human exposure is possible. Is it a toxic soluble, and could it pass through water treatment processes into drinking water? Does it have potential for bioaccumulation in fish, with resultant human consumption. Is the contaminant mainly an atmospheric pollutant? If so, is human exposure through vapor inhalation or are inhalable particulates the main vector for human exposure. A proper study of molecular structure, solubility, volatility and other properties can give excellent indications as to how a monitoring program should be designed.

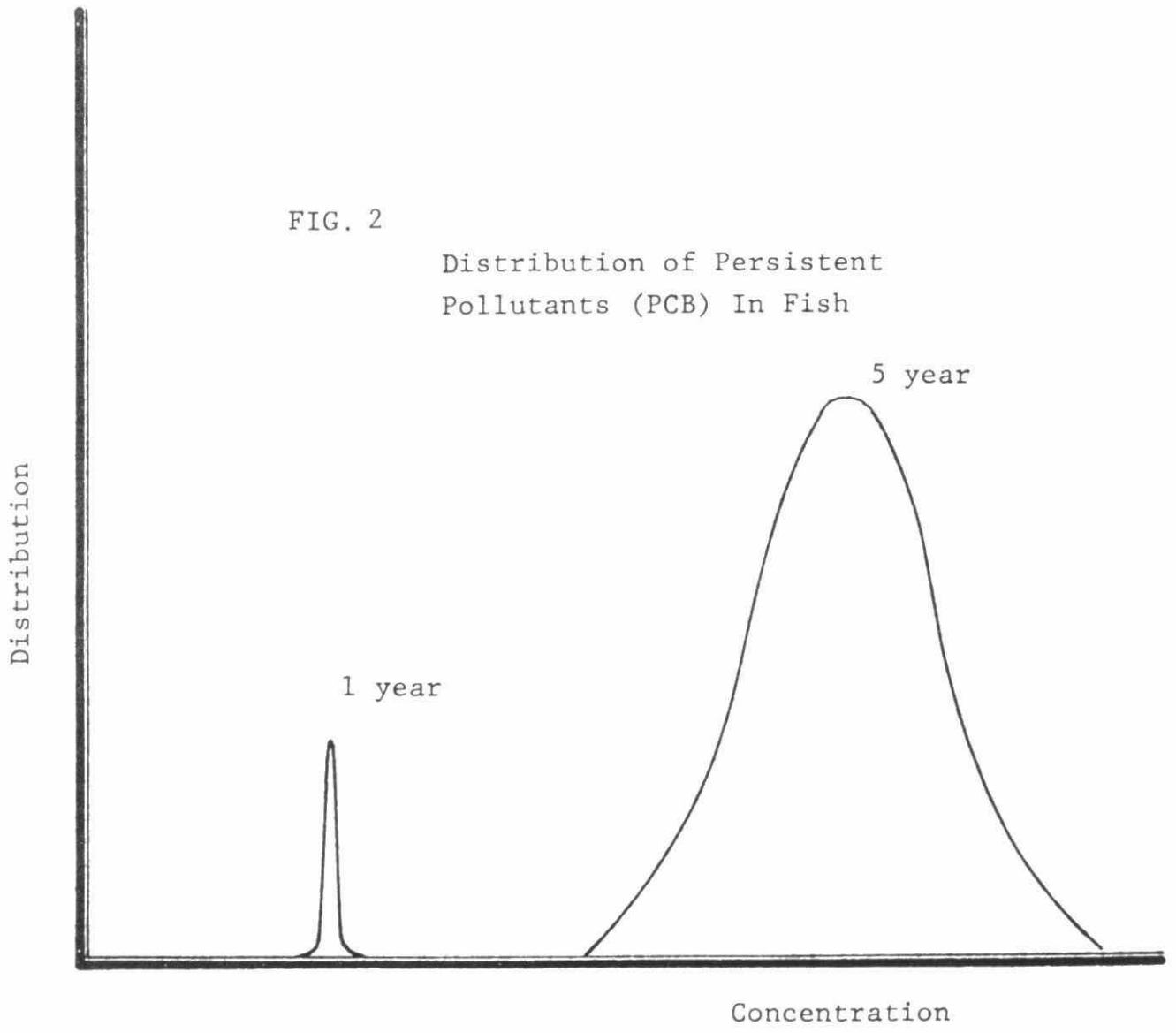
3. Sample Size

In deciding on a sampling program, it is important to know how many samples it is necessary to take to properly represent the distribution of a toxicant in a specific environmental compartment. To obtain a representative sampling of say a fish population experience indicates that 1 or 2 samples are of little use, and at least 20 individuals are necessary to show distribution and mean contaminant levels for the population.

As a fish grows and accumulates organic pollutants, the range of concentrations gradually spreads out as shown in Figure 2. Thus, for very young fish, one or two individuals may well represent the population concentration, but after 4-5 years, especially for top predators, you require

FIG. 2

Distribution of Persistent Pollutants (PCB) In Fish



many more measurements to reflect the population range. Similar problems are encountered in sampling the water in a river for pesticides. Changes in flow rate and suspended, pesticide-carrying sediment can create an order of magnitude difference between samples taken in mid stream or near the bank.

4. Cost

One major criteria in controlling the scope of an organic sampling program is analytical cost. Trace organic analyses are relatively expensive, ranging from $30-$40 for a high volume analysis, such as PCB in fish, up to $1,000-$2,000 for a dioxin/furan analysis on fly ash. Due to the difficulty of trace organic analyses, laboratory capacity must also be considered.

B. SAMPLING AND SAMPLE PRESERVATION METHODS

Once the program is properly designed, suitable sampling and preservation methods must be selected. The methods may take grab samples, integrated grab, or achieve continuous integrated collections. Adsorbent tubes may be used to collect airborne vapors or to extract organics from water. Air filters, water centrifuges or dredges may be used for airborne, waterborne or sediment particulates. Specific collection protocols are available for biological samples.

The samples must be representative of the matrix distribution. The sampling method must be able to provide a large enough sample for adequate method sensitivity. Pesticide scans on water require only 1 litre, whereas dioxin analysis requires 20 litres. Mutagen testing requires up to 500 litres to be concentrated. Hi-Vol sampling of air particulates uses up to 10,000 m^3. The methodology must ensure:

1. Sample integrity during sampling
2. Sample preservation after sampling

1. Sample Integrity

A most important aspect of sampling is the maintenance of sample integrity during sampling. The sample must be protected from losses during sampling and from contamination from the sampling device. Sampling devices must be constricted of inert materials which do not contaminate the sample, do not react with the sample, and do not adsorb materials of interest from the sample. Glass, teflon and stainless steel are the only materials recommended for this construction. Plastics, rubber and lubricants and other synthetics must be avoided. Adsorbent materials such as, Tenax, XAD-Resins for use in adsorption cartridge samplers must be thoroughly cleaned and checked for blanks before use. Particular concern is necessary to ensure that such adsorbents will not react with the sample or sample matrix to cause problems. (Tenax can react with ozone to form artifacts). The sampler must be checked for sampling efficiency. Adsorbent tubes and impingers must be checked for trapping efficiency and capacity at various concentrations of target compound, various typical flow rates, and in the presence of particulates. Grab samplers for volatiles must not cause losses during sampling.

2. Storage and Preservation

Once the sample is taken, it must be properly preserved and stored prior to analysis. Organics have a wide range of chemical and biological activity. Many pollutants can disappear if the sample is left open, exposed to bacteria, sunlight, or heat. Compounds which are stable under anaerobic conditions such as lake bottom sediments may degrade rapidly if exposed to air. Biological activity is also a problem in stored tissue samples. Without the addition of biological inhibitors (mercury salts, acids) even persistent materials like DDT can suffer significant losses during storage in frozen tissue homogenate.

C. SAMPLE PROCESSING AND ANALYSIS

The analysis of a sample for an organic toxicant usually comprises of 4 steps:

1. Isolation in which the target compound is removed from the sample matrix.
2. Clean-up in which the bulk of co-extracting natural organics are removed.
3. Separation in which the remaining mixture of organic pollutants is separated into chemical classes suitable for final analysis step and
4. Analysis in which these clases are separated into individual compounds, and each compound is detected, identified and quantitated.

The first three steps are specifically optimized to remove potentially interfering materials prior to analysis.

1. Isolation

The main purpose of this step is to remove extractable organics (including the target compound) from the matrix. Inorganic analysis allows severe digestion conditions to be used to break down the matrix. However, similar conditions must be avoided for organics due to their reactive nature. Matrix effects are therefore important in organic trace analysis. The compound of interest is often found tied up in the structure of biological tissues, strongly adsorbed to soil surfaces or even incorporated in an inorganic material such as fly ash. Even the recovery of trace from sample matrices such as water can be difficult if the organic is very soluble.

TABLE 2 ISOLATION METHODS

	AIR	WATER	SOLIDS/BIOLOGICAL
VOLATILES	Cartridge Adsorption Impingers Bags	Gas Stripping Head Space Extraction	Extraction Thermal Desorption
NON VOLATILES	Hi-VOL Filter	Solvent Extraction	Solvent Extraction Soxhlet Ultrasonic
	Cartridge Adsorption	Steam Distillation	Shaking
		Resin Adsorption	Steam Distillation

Table 2 illustrates some of the methods used for extraction/isolation of volatiles and non volatile organics from environmental matrices. Volatile organics are collected from air using adsorbent cartridges, impingers, or gas bags. They can be recovered from liquid samples by gas stripping (purge/trap) head-space sampling, or sometimes by solvent extraction. Efficiency of volatiles removal from liquid scan be very dependent on the makeup of the liquid sample since purging or vaporization is slow and inefficient from very viscous liquids, or liquids with high solubility for the target compound. Volatiles in solids or biological samples are frequently recovered by thermal desorption into a gas stream, from which they are further trapped. This process can alos be relatively inefficient due to slow diffusion and mass transfer out of the bulk of the matrix.

"Non volatile" or "semi-volatile" organics are recovered from most matrices by solvent extraction. Air samples often contain these materials in vapor state or adsorbed on particulates. The vapors are trapped on filters or cartridges or in impingers, the particulate adsorbed materials are collected in Hi-Vol filters. Organics are then recovered from these devices using solvent extraction.

Isolation from water or other liquids is achieved through direct solvent extraction, or adsorption of the organics on a resin cartridge followed by solvent extraction.

Particulate samples, e.g. soils, sediments and fly ash often provide the most difficult matrix for organic extraction. Soils, sediments, fly ash have strong adsorptive affinities for organics and bind them very strongly. Quanitative recovery is frequently impossible, even after vigorous pretreatment (acid treatment, ultrasonic) and extended solvent extraction spiked. Samples used to check organics recovery seldom give a real measure of recovery for natural residues, since the latter are

thoroughly incorporated into the matrix structure. Methods used include continuous extraction with hot mixed solvents, ultrasonic solved extraction and even steam distillation. Extraction of biological samples (plants, fish, insects, etc.) usually requires disruption of the cell structure to release organic pollutants. Grinding, ultrasonic disruption, cold acid digestion, each followed by solvent extraction are frequently used for isolation of organics from this type of sample.

2. Cleanup

Cleanup steps are designed to remove the bulk of the co-extracted natural organics from the sample extract. In the case of volatiles analysis this is frequently neither necessary, nor practical due potential losses during handling. So-called "Non Volatile" organics, on the other hand, frequently cannot be analysed without thorough cleanup.

A range of cleanup techniques are available. Table 3 lists some of these techniques.

Liquid/liquid partition between appropriate solvents can be used to leave natural organics in one liquid phase whilst transferring compounds of interest to another phase or solvent. Adsorption chromatography places the sample extraction on a column of adsorbent such as florisil, alumina, charcoal. Selective solvent elution is then used to leave behind the natural organics whilst recovering the compounds of interest in the eluate.

Steam distillation can be used to remove smaller (PCBs, pesticides) organics from relatively non volatile natural fats and waxes. Molecular size separation (gel permeatian) separates and cleans up by a size fractionate method. This type of cleanup is illustrated in Figure 3. The bed of gel material has pores of a very specific size and shape. The sample extract is placed on the column and pumped through using a solvent stream. Large molecules cannot penetrate the small pores in the packing, and are rapidly carried through the system by the solvent flow. Smaller molecules penetrate the pores and are slowed down, effecting a separation.

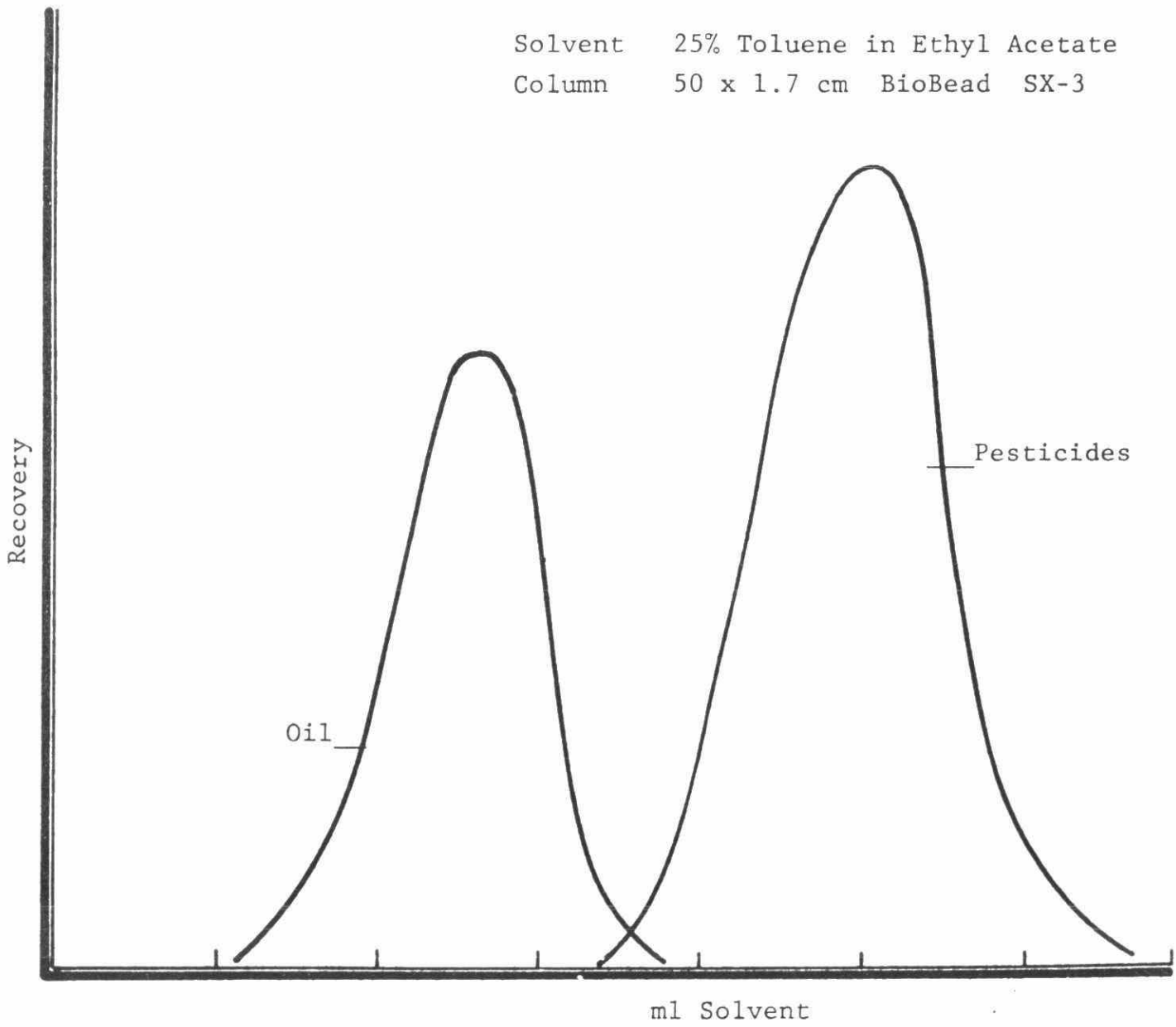
TABLE 3 CLEANUP METHODS

LIQUID/LIQUID PARTITION	
ADSORPTION COLUMN CHROMATOGRAPHY	Florisil Alumina Charcoal "Sep-Pak"
STEAM DISTILLATION	
MOLECULAR SIZE SEPARATION	Gel Permeation

FIG. 3

GEL PERMEATION CLEANUP OF
PESTICIDES IN FISH OIL

Solvent 25% Toluene in Ethyl Acetate
Column 50 x 1.7 cm BioBead SX-3



3. Separation

The mixture of organic pollutants left in an environmental sample extract after cleanup is still complex. It often contains hundreds of discrete compounds, ranging in amounts from micrograms to picograms. These need to be further separated into simpler chemical groups or classes suitable for final analysis. Table 4 lists some of these techniques used including chemical reaction, adsorption column chromatography, and various high resolution liquid chromatography methods.

An example of this type of problem is the analysis of fish samples for dioxin.

Great Lakes fish contain PCBs up to 50 ppm, chlorinated pesticides up to 5 ppm, mirex, chlorodiphenyl ethers, methoxy, chlorobiphenyls. Dioxins must be analysed in the complex mixture of chlorinated compounds in amounts of only a few picograms. Many of these compounds cause severe interferences in the analysis of dioxins at the parts per trillion level and must be separated prior to analysis. Because of the difference in concentration at least 99.9999% of the interferences must be removed. Table 5 shows the type of cleanup and separation necessary to achieve this.

The sulphuric acid column removes oils and fats, etc.

The first alumina column removes the residual natural organics, pesticides and 80% of the PCBs.

The silver nitrate column removes sulphur and unsaturated compound.

The second alumina removes 19% more PCBs and other chlorinated compounds. Reverse phase HPLC removes the last of the PCBs, and permits some fractionation of dioxins. The normal phase HPLC permits a fraction with approximately 4 TCDDs including 2,3,7,8-TCDD to be recovered. This is now suitable for analysis by high resolution capillary GC/MS.

TABLE 4 SEPARATION METHODS

CHEMICAL REACTION

ADSORPTION COLUMN CHROMATOGRAPHY

HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

TABLE 5 DIOXIN CLEANUP SCHEME FOR FISH

STEP	PURPOSE
Extraction	
Acid Digestion	Breaks Matrix Permits Cleanup
Cleanup	
H_2SO_4/Silica	Removes Fats & Oxidizables
Separation	
Alumina	Removes Pesticides + 80% PCBS
Ag No3 (Silica)	Removes Sulphur Compounds and Unsaturates
Alumina	Removes 20% PCBS
Reverse Phase HPLC	Removes Residual <1% PCBS/Fractionates Dioxins
Normal Phase HPLC	Final Dioxin Fractionates

4. Analysis

Isolation, cleanup and separation have now hopefully produced a suitable extract for final analysis. The final analyses of these purified extracts is normally carried out using some form of instrumental measuring device. Whilst a wide range of instrumental systems are available for this step, I will only cover the use of a few of the more common systems in this presentation.

Trace organics analysis system in MOE's laboratory usually comprise of a final separative step such as gas or liquid chromatography coupled with some type of detection and quantitation device - the detector. Table 6 outlines common separation and detection steps for trace organic analysis.

Separation

The separative step has the purpose of resolving the remaining mixture and presenting individual compounds to the detection system for identification and quantitation. These steps are usually some form of chromatography. Chromatography, whether it is gas or liquid chromatography uses a mobile fluid - (gas or liquid) to carry the sample mixture through a tube containing an adsorbent materials, which may be liquid or solid. Individual compounds interact differently with this material and are retarded to different extents, thus causing a predictable separation. The retention time of any individual compound under controlled chromatographic conditions is extremely reproducible and is normally used as one of the main identification tools in routine trace organics monitoring.

Classical GLC and HPLC used relatively low resolution short (1 ft. - 6 ft.) columns. These are packed with porous support material carrying a stationary liquid phase, either coated or bonded to its surface. It is this liquid which caused the selective separation effect. The need for more reliable trace analysis has caused analysts to move to systems with much higher resolution -capillary columns. These narrow, long (10-100 metre) tubes have the adsorbent material (liquid) coated or bonded to their inner surfaces. Resistance to fluid flow is low, and resolution is greatly enhanced.

TABLE 6 ANALYSIS METHODS

SEPARATION STEP		DETECTION STEP		
		General	Selective	Tuneable/ Structural
Gas Chromatography	*	Flame Ionization Thermal Conduct Photoionization	Electron Capture Hall Flame Photometric Thermionic	Mass Spec FT/IR Microwave/Plasma
HPLC		Refractive Index Flame Ionization	Electron Capture	Mass Spec Infra Red Ultra Violet Fluorescence

The need for and use of this enhanced resolution can be seen in this analysis of PCBs in ambient air. The sample is collected on florisil cartridge, extracted, cleaned up and chromatographed using Electron Capture GC. The original GC method used and packed column chromatography gave the trace shown in Figure 4A. There are still a large number of interferences in this area of the chromatogram. The more recently introduced capillary columns on the other hand allow us to resolve many of the interferences and get the chromatograph in Figure 4B. Use of 2 capillary columns simultaneously allows even more interferences to be resolved -some on one column - some on another. This "Dual Simultaneous Capillary" method for PCB in air analysis was developed at MOE by Dr. Eugene Singer of Air Resources Branch. The effect of its use on the result of an analyses for PCBs in air is shown in Table 7. PCBs by packed column gave high results. A single capillary column gave a lower result, and dual simultaneous capillary column analysis gave a lower result still.

Detection

Once the final extract has been separated by GLC or HPLC into its components, a detection system is needed. A chromatographic detector should have a stable, and predictable response which is proportional to varying concentrations of organics, so that target compounds can be quantitated. Some degree of detector selectivity or specificity is also desirable to improve identification capability. The Table 6 shows some of the detectors in common use for GLC and HPLC. A brief description of the modes of operation of some of the more common detectors can be found in Appendix I.

General detectors respond to most organics and can be used to give a total picture of compounds emerging from the chromatograph. However, they have no specificity or selectivity, and thus can give no structural information on the identity of a peak.

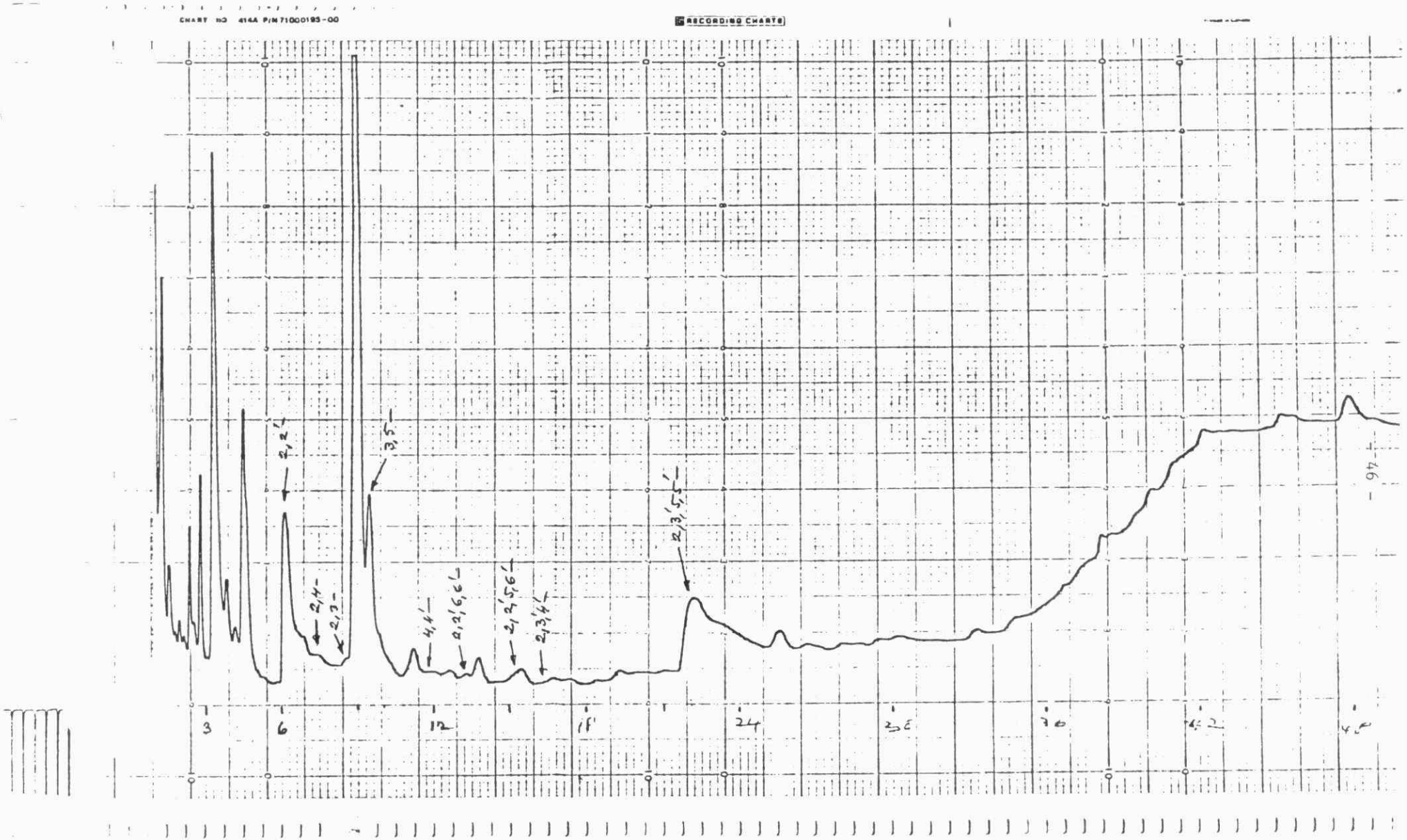


FIG.4A PCB ANALYSIS OF AIR SAMPLE
PACKED COLUMN

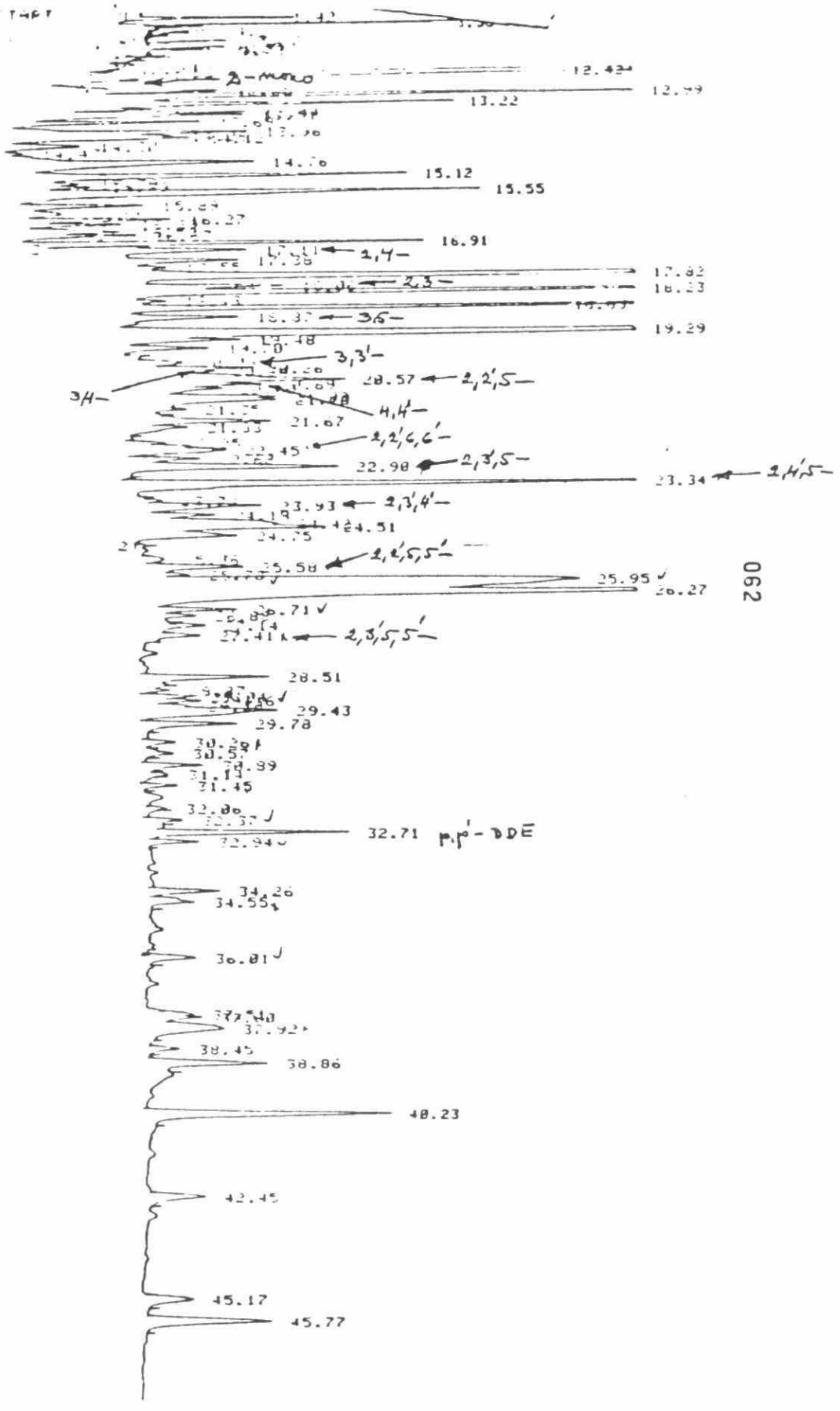
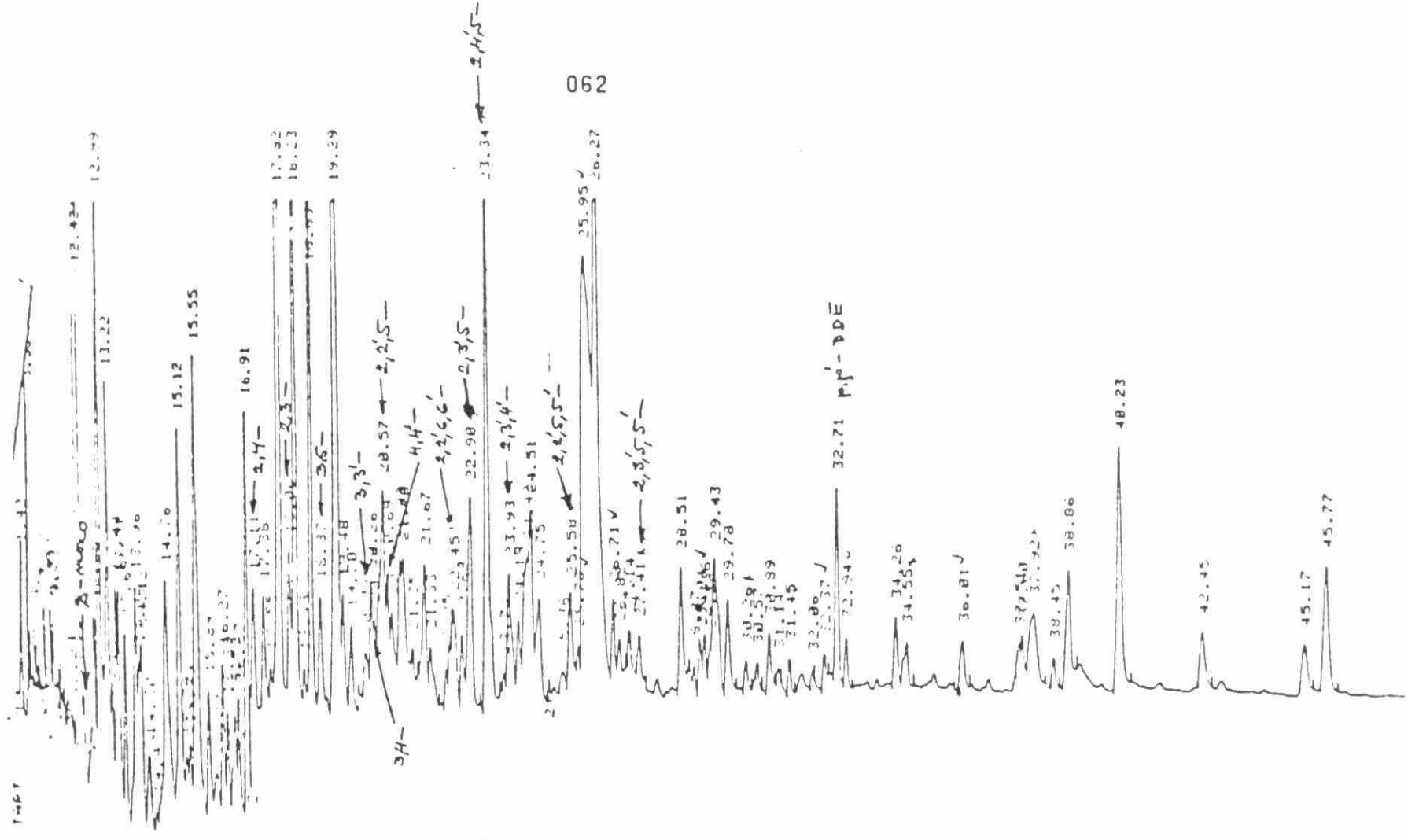


FIG. 4B PCB ANALYSIS OF AIR SAMPLE
CAPILLARY COLUMN

TABLE 7 COMPARISON OF AIRBORNE PCBS MEASUREMENTS (ng/m^3)

	SITE	PACKED COLUMN	SINGLE CAPILLARY	DUAL CAPILLARY
NA	S1-11-1	317	11	3.3
NA	S1-12-17	44	1.0	0.7
MO	S1-R1-4	33	2.9	0.1
SAR	S1-11-2	1615	4.3	2.1



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MO	S1-R1-4	33	2.9	0.1
SAR	S1-11-2	1615	4.3	2.1

Selective detectors - electron capture, flame photometric, thermonic, on the other hand, respond only to compounds containing specific elements or classes of element. Thus, a phosphorus selective flame photometric detector is useful in selectively detecting organophosphate pesticides.

Tuneable, structure specific detectors such as, mass spectrometer, or Infrared, Ultraviolet or Fluorescence Spectroscopy can give information on the actual molecular structure of a compound and, in the case of Mass Spectrometry, can actually fully identify this structure by fragmenting it and examining the fragement patterns. The Mass Spectrometer, which gives unique identities to peaks, would appear to be the system of choice for accurate qualitative work for all chromatography systems. Unfortunately, mass spectrometers are still extremely expensive in comparison with other detectors, they are difficult to automate and operate for routine monitoring, and are less reliable in terms of quantitative response. Normal monitoring programs for trace organics usually use one of the less specific but more routinely applicable detectors such as, Flame Ionization, Electron Capture for GLC, Ultra Violet adsorbance or fluorescence for HPLC. We thus have to sacrifice some degree of specificity for practicality. This type of compromise is frequently necessary in the selection of many steps in the organic analysis process. The compromises made imply something other than perfection in the design of the sampling and analytical method. The variability introduced by these compromises lead us to our next concern - the quality of data produced by these methodologies.

D. DATA QUALITY ORGANIC TOXICANT ANALYSIS

This outline of the difficulties encountered and compromises necessary in an environmental analysis for organic toxics shows that the complexity of the process can introduce many variables into the final measurement. The degree to which these variables cause differences between the "true value" and the measured result, and variations in this measured result is what has to be determined and controlled in a Data Quality Assurance program. Such a program is particularly important when the data produced is to be used to assess potential human health hazards. The ideal situation for an environmental analysis is when the final result matches exactly the original concentration of target compound. This requires:

(a) 100% recovery of analate during sampling and analysis
(b) Complete resolution of analate from interferences
(c) Unequivocal identification of analate
(d) Quantitation of analate against a completely stable and predictable instrument response curve, with a unique response to the target analate

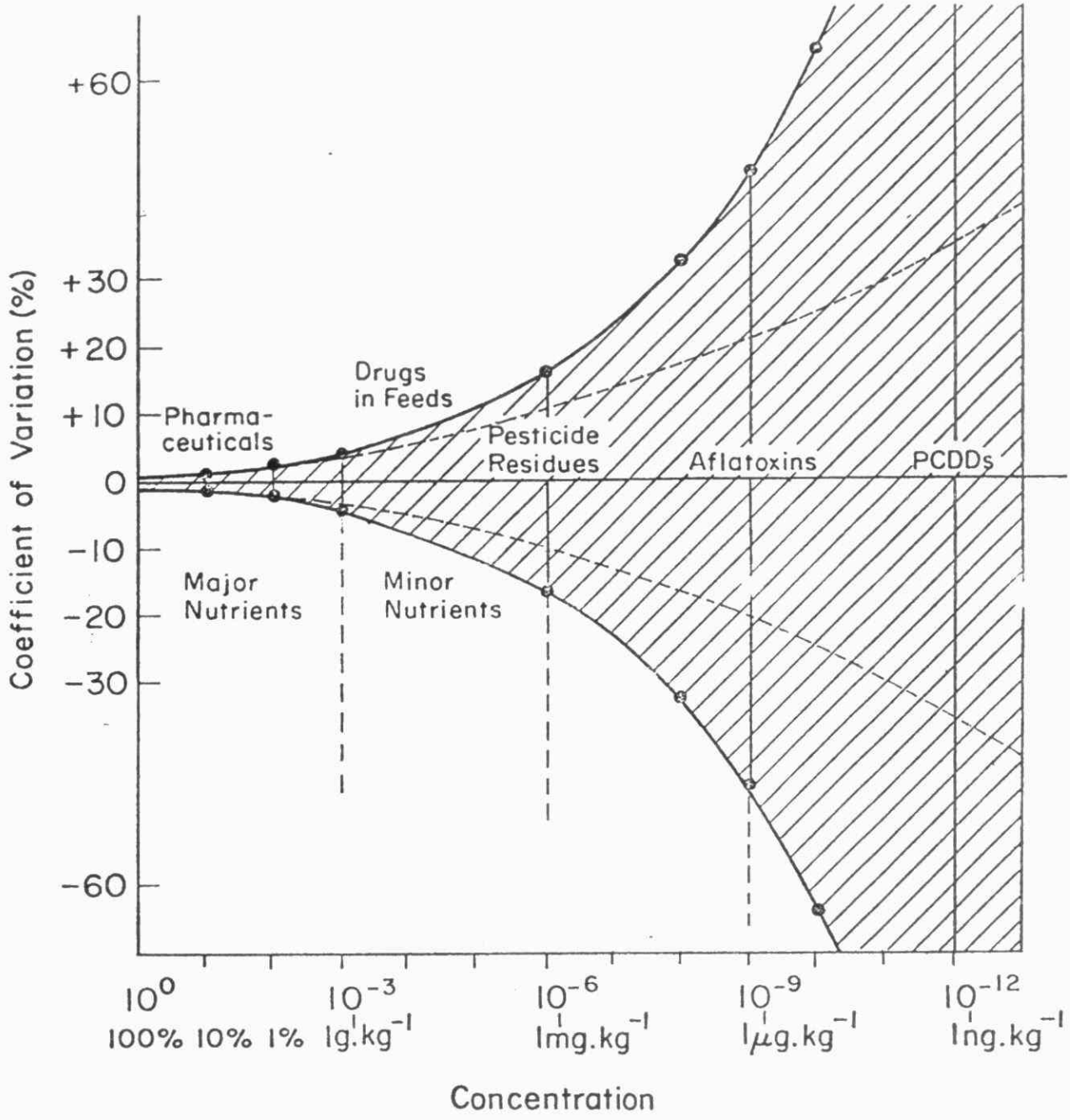
In reality, none of these objectives are completely achievable. The reasons for this are partly unavoidable since they are due to the properties of the sample and target compounds. Partition coefficients, volatility, irreversible adsorption, sequestering by biomaterial, etc. make it all but impossible to recover 100% or even an exactly reproducible quantity of a naturally incurred toxic from an environmental sample. However, part of the variability, and uncertainty of identity is introduced because of conflicting demands placed on the sampler/analyst. A certain amount of data is required for program delivery. If the optimum recovery, identification and quantitation steps were used for obtaining this data, (multiple extractions, partitions, ultra slow evaporations, mass spectrometric identification and qualitation, labelled spiking of each sample, etc.) the cost of a single analysis would be prohibitive, and the laboratory capacity reduced, 2-3 analyses per day. In practice, compromises are made to permit rapid sample processing,

automation of instrumental analysis and data interpretation, etc. If a phenol analysis took 2-4 days and cost more than $500; it would be of little use for monitoring.

To reduce uncertainty caused by the above two sets of variables, the analyst is continuously working on improving recovery, precision of measurement and certainty of identification, whilst retaining capacity for reasonable volume of analysis. Other demands on the analyst have included a need for rapid reductions in detection limits - adding to these difficulties listed above.

Whilst we are always trying to reduce the uncertainty, it is essential that we know the precision and accuracy of data being produced by current methodologies. We must have reliable Data Quality Assurance protocols. Quality Assurance protocols for trace organics analysis are, of course, built into the sampling and analytical methodologies. Instrument calibration, analysis of field and laboratory blanks, spikes and duplicates are routinely carried out. However, many of the accepted Quality Assurance protocols -analysis of duplicates, analysis of standard reference materials, analysis of spiked samples, etc. have definite drawbacks in the case of trace organics work. Duplicate sampling of the environment for organics is difficult due to the variability discussed in the section on sampling. Split sampling is not always reliable, since, for example, in sampling water, organics can rapidly deposit on the bottle walls. The whole sample must therefore be extracted to recover all organics present. Standard reference materials are seldom available for organics in environmental matrices, and even when they are it is seldom known whether the "certified" analysis provided is a real measure of total analate concentration in the sample is merely the best result obtainable by current methodology. Similarly, a recovery figure obtained by spiking environmental samples usually gives an inflated idea of the recovery of the same analate naturally incorporated into the sample. Not withstanding these limitation, Quality Assurance using these techniques is an integral part of trace organics monitoring.

FIG. 5



Interlaboratory coefficient of variation as a function of concentration

Figure 5 is a plot of the type of precision obtained by laboratories participating in a range of AOAC interlaboratory check sample programs for trace organics and drugs in foodstuff and biological samples. I should emphasise that this was produced from data supplied by proven reliable laboratories, and not an example of poor performance. Coefficient of variation at the parts per billion level is ± 40% and as we approach the parts per trillion sensitivity now being required of analytical method, the coefficient becomes very large. Since this graph was drawn up in 1980, significant gains have been made in precision and accuracy at the parts per trillion level analysis (dioxins). The second pair of dotted lines represents a rough estimate of the overall improvement in analytical precision which has resulted from rigorous method development and quality control procedures introduced for ppt level analysis such as, dioxins in fish. These improvements have been achieved at a significant cost in terms of analytical output and cost (up to $500 per sample). Even with these improvements, recovery of isotopically labelled dioxin standards at the 20 ppt of 60%, with a variation of 20% is regarded as very acceptable. Inter-laboratory studies on 2,3,7,8-TCDD in fish can now achieve a C.V. of approximately ±40% at 20 ppt. So that even with the best of analytical techniques, as methodology is pushed further and further significant variability must be anticipated. This is obviously going to have major impact in the way data is interpreted.

DATA INTERPRETATION

When a program designer is planning an analytical monitoring program, it is essential that he notify the analyst of the use to which the data will be put, and the quality of data required. If the designer requests or agrees to an analytical procedure which produces "rough" data such as head-space analysis for volatiles using unsophisticated packed column GC instrumentation, he must not expect to be able to use the data so obtained for drawing accurate qualitative and quantitative conclusions. Even sophisticated GC/MS identification methods must not be used for quantitative projections, unless the whole system has been precisely calibrated for the target compound. Data must be evaluated only in light of the statistical soundness of the sampling program design.

PROGRAM FEEDBACK

Program design for trace organics monitoring must be a dynamic process. Problems which show up in one year's monitoring must be clearly recognized, solutions defined, and the results of this definition fed back into program design for the next season. Especially in the case of a newly recognized toxicant, monitoring results should not be used for control and regulation until such time as experience indicates all the "bugs" in the sampling and analysis protocols have been recognized and eliminated.

CONCLUSION

With all these caveats it may appear to you that trace analyses for organic toxics are of little value. This is, of course, not true. With proper care in program design, analysis and data validation, trace organic analysis can give extremely useful information on the potential for human health effects of organic toxicants in the environment.

APPENDIX I

Detector Operation

Flame Ionization

This general organics detector consists of an air hydrogen flame, which burns the effluent from a GC (or HPLC). As organics emerge from the column, their commbustion increases the ion concentration in the flame. This change is monitored by a pair of polarized electrodes and produces the detector signal.

Electron Capture Detector

A radioactive foil emits ρ radiation and ionizes the carrier gas. Polarized electrode monitor this level of ionization. Molecules containing electro negative atoms (Halogens, Phosphorus, Oxygen or groups ($-NO_2$)) can capture electron formed during the ionization process and reduce the level of ionization. The electrodes monitor this reduction producing a signal.

Flame Photometric

The GC effluent is burned in a hydrogen rich flame. Molecules containing elements such as, phosphorus or sulphur form species in the flame which emit characteristic wavelength radiation. By the use of selective interference filters, and photomultiplier tubes, these emission lines are monitored uniquely and produce a chromatogram representing only compounds containing these elements.

UV, IR, Fluorescence Spectrometers

Specific molecular structures (aromatic rings, carbonyl groups, PAH structures, etc.) can adsorb radiation (UV, IR) or emit (fluorescence) radiation of a characteristic wavelength. Spectrometer

systems can be tuned to expose the effluent from a chromatograph to visible, ultraviolet, or infra red light, and to monitor for specific wavelength adsorbance or emission characteristic of a given molecular structure.

Mass Spectrometer

In this system, organic molecules emerging from the chromatograph are bombarded with electron in an evacuated "source". The molecules produce a pattern of fragments whose masses and distribution is uniquely characteristic of the original molecule. The mass spectrometer separates the fragments by mass and accurately measures their mass and intensity. The "mass spectrum" of the compound can then be identified by interpretation from theoretical principles of fragmentation, or, more commonly, compared with a library of known mass spectra on a computer system. Specific compounds or classes of compounds can be monitored by adjusting the mass spectrometer to monitor one or more ions specific to that compound.

Health Effects of Environmental Pollution Seminar

October 27, 1983

Transport of Toxicants: Distribution and Fate in the Environment

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INTRODUCTION.

In this paper we review briefly some aspects of the fate and behaviour of environmental contaminants. There are four categories of information that are needed before we can fully describe their behaviour, and which are useful (and possibly even essential) for implementing adequate controls. These are:

(1) Sources: emission rate and location.

(2) Partitioning properties between the various environmental media of air, water, soil, sediment and biota.

(3) Reactivity in processes such as hydrolysis, biolysis, photolysis, oxidation in all media.

(4) Transport rates between media such as soil and air, water and sediment or biota and water.

A fifth activity is to take these data and synthesize them into an overall assessment of contaminant fate or a "behaviour profile" in which the dominant media of accumulation are forecast, the principal reactions identified and transport routes elucidated. It may even be possible to estimate mean

concentrations in various media such as air and water and in foodstuffs, notably fish.

This process proves to be quite difficult and expensive and only for a few compounds is there a comprehensive and clear understanding of environmental fate. Part of the difficulty lies in the number of contaminants. Most jurisdictions (including Ontario) have their lists of nasty substances, some runningto many hundreds. It is clear that to make progress in tackling the vast problem of assessing and controlling all these substances we must seek out the generalities which apply to them all. There is no prospect of any one compound being afforded the attention which has been devoted for example to phophorous as an eutrophication agent. The PCBs alone comprise 209 compounds! The environment is also very complex (Fig. 1) and variable in space and time.

Some of us have become convinced that the answer lies in developing general models of environmental fate and behaviour which can be used for allsubstances; but into the model we insert the peculiar properties of each substance. If this can be accomplished it may be possible to generate estimates of "exposure" and then address the more difficult problem of assessing the toxic effects on a diverse population. But here we consider only the simpler problems of assessing environmental fate.

In some respects our problem is similar to that faced by the income tax agencies. They must treat some 10 million individuals, each with their own income, family circumstances

and excuses for deductions. Only with the aid of computer models can they adequately assess the multitude of information. Some believe they do the job too well! Perhaps our aim should be to treat toxic substances like tax-evaders with the same enthusiasm, vigour and modern computing methods.

Sources.

There are at least four source classes, chemicals of commerce which have escaped or been discarded (many of which are designed to be biologically destructive to "pest" organisms); substances which have been synthesised inadvertantly either industrially (eg. dioxins) or in the environment (eg. haloforms); substances present in waste streams of air, water or solid wastes especially sludges; and finally substances resulting from the recovery and processing of minerals and fossil fuels.

Some are indestructible, notably the metals; thus they will remain indefinitely until advected by water flow or (less desirably) by incorporation into harvested foods such as fish or vegetation. Metals can thus cause severelong term problems, expecially in soils that have received sludges containing metal and which may be used for agricultural purposes. Of particular concern are those metals which form organic compounds such as methyl mercury and which may bioconcentrate. Many organic compounds are "recalcitrant" to degradation by biological or chemical methods. Notable are the humic or ligneous organics which are fortunately biologically quite inert and the organochlorines which are usually very stable, fat soluble, water

insoluble and inert. Examples are the chloro-benzenes, -naphthalenes, -biphenyls and -alkanes. These compounds are anthropogenic or "man made"; thus enzyme systems which have evolved over the eons of time since life appeared on Earth may not have developed the capability of breaking them down (or mineralizing them) to innocuous CO2 and chloride ion. Other non-chlorinated organics such as phthalate esters or non-linear alkanes may also be slowly degraded. There is emerging a viewpoint that chemicals of commerce, whether they be detergents or transformer oils, should be environmentally degradable or non-persistent since inevitably they will be released or spilled. At least their use should be carefully controlled. Perhaps there is a compelling incentive to ensure that their degradation is assured, industrially or environmentally. Their entire "cradle to grave" journey must be assured. Even if they are apparently non-toxic, their accumulation in biological systems is inherently a cause for concern since our ability to predict subtle toxic effects is certainly less than our ability to synthesize and exploit new chemicals. There is of course a large group of "non-persistent" or degradable substances which will react fairly rapidly in the environment and while possibly exerting a toxic effect will be destroyed in weeks or months. They should be accorded less scrutiny. We are still not yet competent in categorizing these sources and chemicals into degrees of nastiness.

Partitioning.

It is a well known principle of physical chemistry that a

substance will partition between any two phases (e.g. oil and water) at different concentrations. There is a growing realization that there are three principal media: air, water and "organic". The organic phase includes biota, lipids and organic matter present in sediments. This can be displayed conveniently on the triangular diagram as suggested by Slimak (Fig. 2). Freons obviously partition into air, phenols into water, and dioxins into organic matter. Particularly troublesome are metals which undergo chemical speciation changes and thus "switch' their partitioning preferences. Mercury is the classic example but there are others such as tin and lead. There is a compelling incentive to gather accurate data on these properties, notably water solubility, vapour pressure and octanol-water partition coefficient (Kow). From such data it is often possible to calculate the concentration ratios which will be approached in the environment. For example if Kow of a substance is 1 million, a sediment organic carbon-water partition coefficient is probably about 400000 and a fish-water partition coefficient about 50,000.

Reactivity

There are many reactions which a substance may undergo, particularly hydrolysis, photolysis, oxidation and biolysis or biodegradation. The rate of reaction depends on the chemical nature of the substance, on the environemental medium (e.g. air or water) and on environmental conditions such as temperature, pH, microbial population, solar radiation and the presence of other reactive chemicals such as ozone. It is important to identify the principal reactions or degradation processes and

their rates or "half lives". Obviously a substance with a half life of 4 hours is of much less concern than one of 4 years. Estimation of "persistence" is thus a critically important component of environmental fate assessment. In some cases the substance unfortunately tends to partition into a medium in which reactivity is low. For example a substance may be quite photodegradable but may be trapped in dark sediments.

Transport.

It is only in recent decades that the full potential for transport or migration between compartments has been recognized. There have been some surprising migrations such as DDT volatilizes appreciably from soil or water despite its low vapour pressure. Much is now known about uptake and release between fish and water. The most difficult problem is predicting transfer between sediments and the overlying water column. A capability is, however, emerging for calculating these transport rates and with some luck the surprises should be less frequent. We have now no excuse for mistakes of the magnitude of DDT or PCBs or mercury.

In some cases such as air-water exchange it appears that there may be several simultaneous transport processes, some complementary, some competing (i.e. in opposite directions). These are identified in Figure 3.

Models.

It is now apparent that two types of models are needed, evaluative or hypothetical environmental models and real environmental models. Figure 4 shows a typical evaluative

environment consisting of defined volumes of air, water, soil etc. A chemical can be introduced into this "unit world" and its behaviour calculated. A "behaviour profile" can thus be obtained and the dominant media of concentration and reaction identified. It is hoped that these profiles give a valid indication of real behaviour. Examples are the fugacity models and the EXAMS model of EPA. Figure 5 gives typical model outputs. Unfotunately these models are not easily validated but they are simple and very useful.

Real models have the great advantage that they can be validated. Unfortunately they are often very complex mathematically and apart from the modeller, few have the inclination to take the time to understand them fully. Figure 6 gives an example of an output of a real model for a pond using our QWASI (Quantitative Water Air Soil Interaction) model.

These models usually generate statements of the type "the mean concentration in sediment is 4 ng/g". But in the real environment there is usually a distribution of concentrations with, for example, a few sites having values greater than 40 ng/g, more having values of 10 to 40, and most having values of 0 to 1 ng/g. This can be expressed as a concentration distribution function as illustrated in Figure 7. Contour plots can be obtained as shown in Figure 8 and compared with real monitoring data.

Conclusions.

There has emerged in recent years a new branch of environmental science which seeks to describe the behaviour of

chemicals as they partition, react and migrate through the environment, concentrating in various media to differing extents. This science, which has been termed "chemodynamics" or "environmental toxicology" is advancing rapidly and it is expected that in the next few years there will be available proven techniques for testing chemicals and assembling the test data into models which can be used by environmental regulatory agencies to predict how these chemicals will behave. Our aim in the next few years should be to refine our techniques, prove them by application to real problems such as those faced by the Ministry and build up confidence that we can adequately predict the environmental fate of chemicals. It then becomes possible for us to address the next, and more difficult problem of assessing the likely toxic effects.

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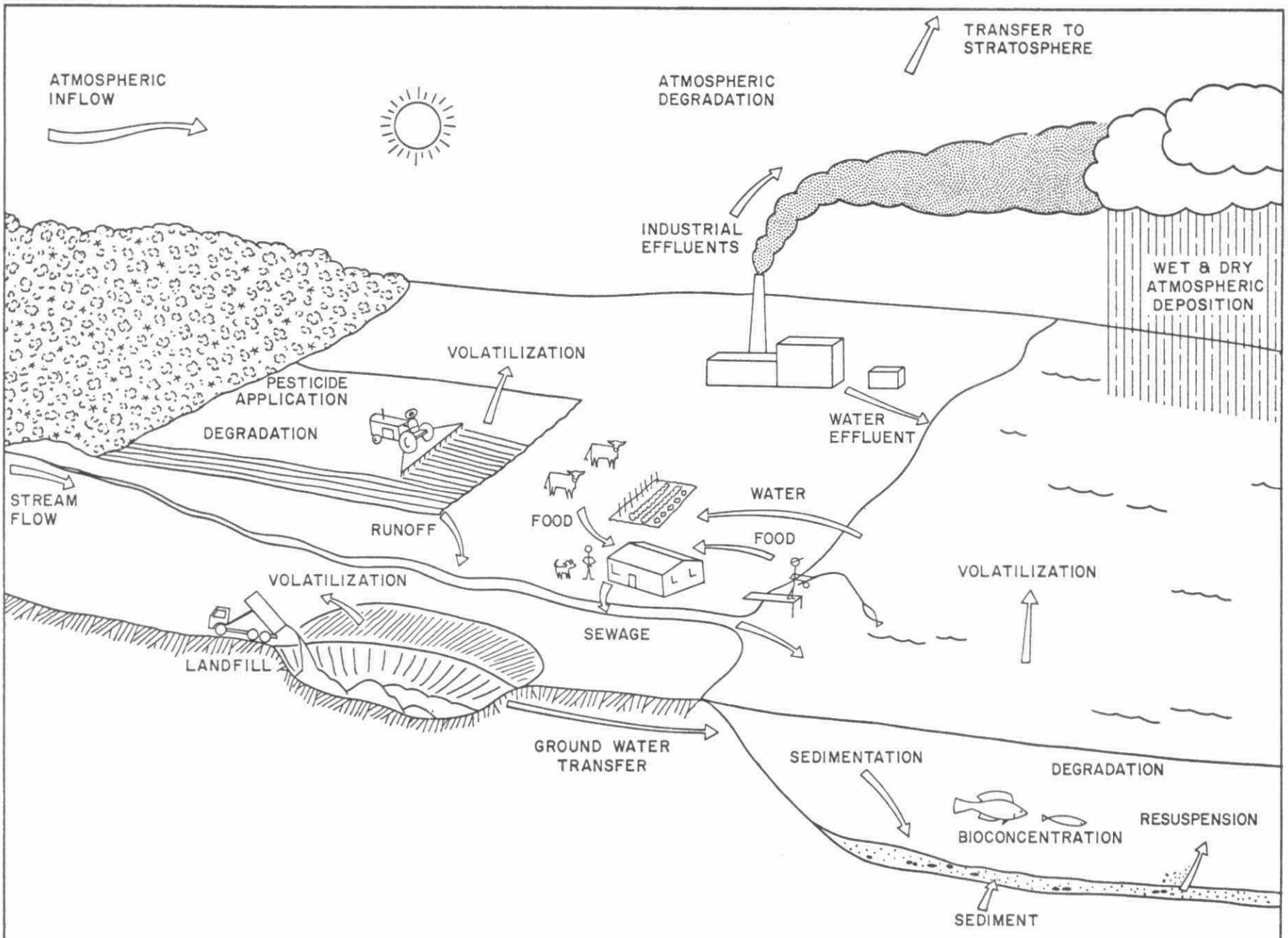


Figure 1. The Environment

1. Benzene, toluene, and mono-, di- and trichlorinated ethanes and ethenes
2. *a*-Endosulfan
3. Endosulfan sulfate
4. Phenol
5. Benzidine
6. Dimethylphthalate
7. Diethylphthalate
8. 2,4-Dichlorophenol
9. PCB-1254
10. TCDD
11. Benzo[a]pyrene, chrysene, *bis*-(2-ethylhexyl)phthalate, DDT, DDD, DDE

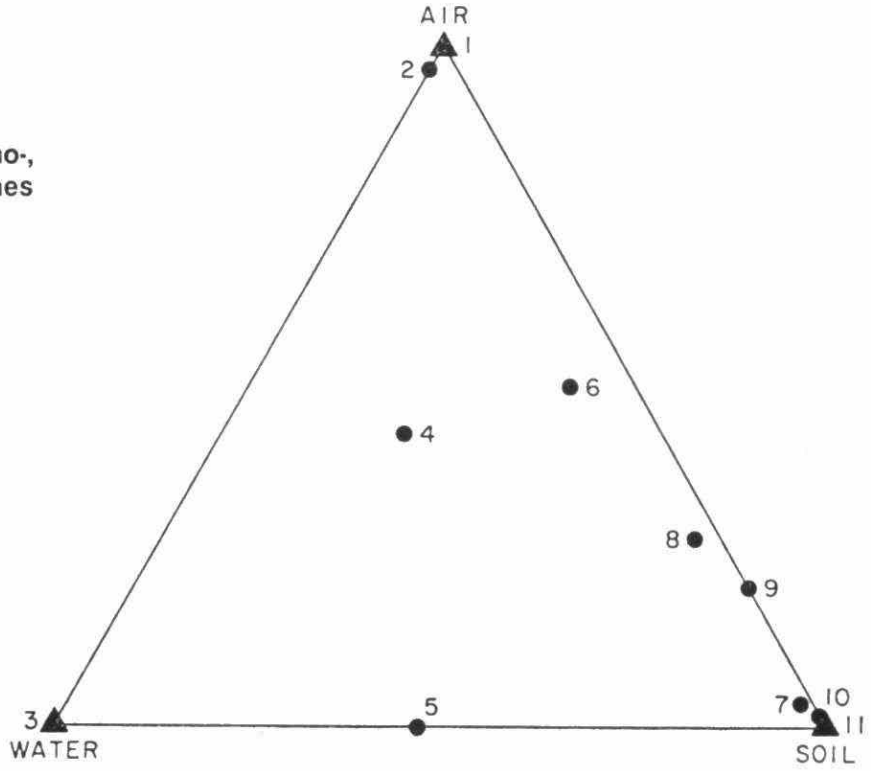


Figure 2. Equilibrium distribution of several priority pollutants between environmental media (percentages).

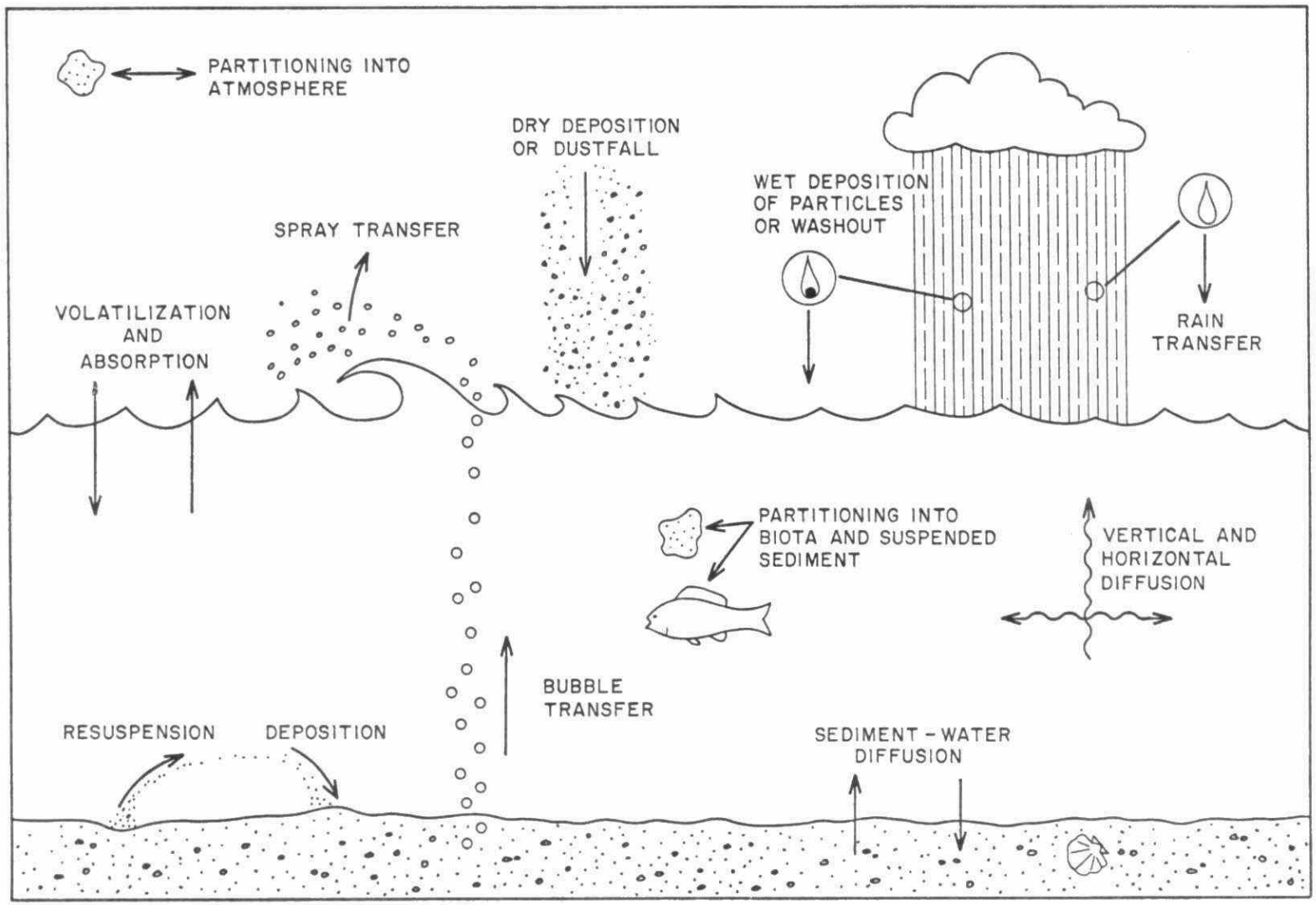


Figure 3. Air-water exchange processes

FIGURE 4.
Unit world volumes (V m^3) and areas (A m^2)

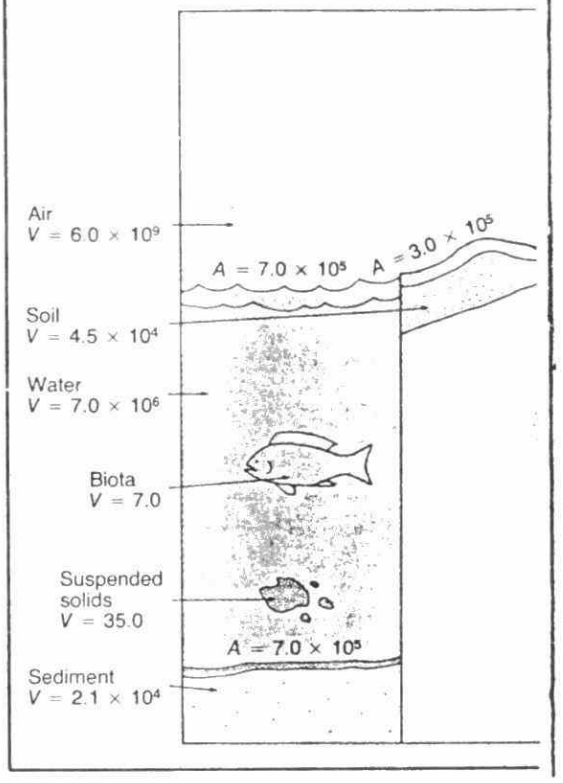
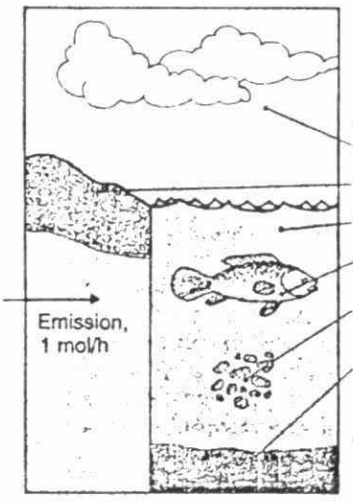


FIGURE 5a.
Illustrative Level II calculation with no advection



	Amount, mol	Concentration, mol/m^3	Reaction, mol/h
Air	45.61	4.56×10^{-9}	0.502
Soil	1.93	2.15×10^{-4}	0.031
Water	26.38	3.77×10^{-6}	0.422
Biota	0.0025	7.10×10^{-4}	0.0002
Suspended solids	0.0150	4.29×10^{-4}	0
Sediment	9.01	4.29×10^{-4}	0.045
	82.95		1.000

Fugacity = 1.131×10^{-5} Pa

Persistence = 82.95 h

FIGURE 5b
Level II behavior profile with a common fugacity

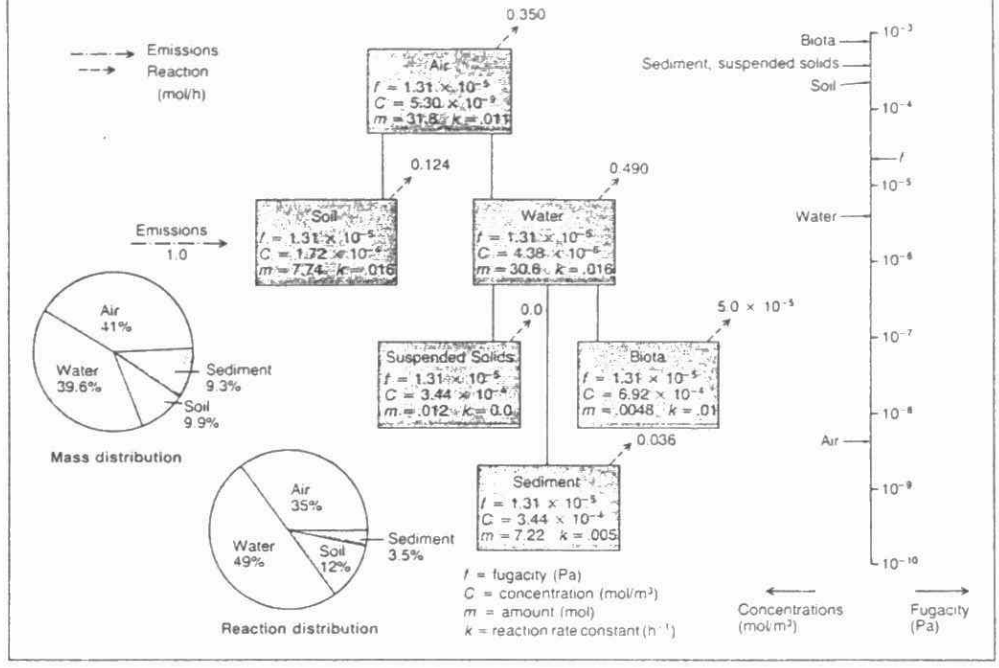
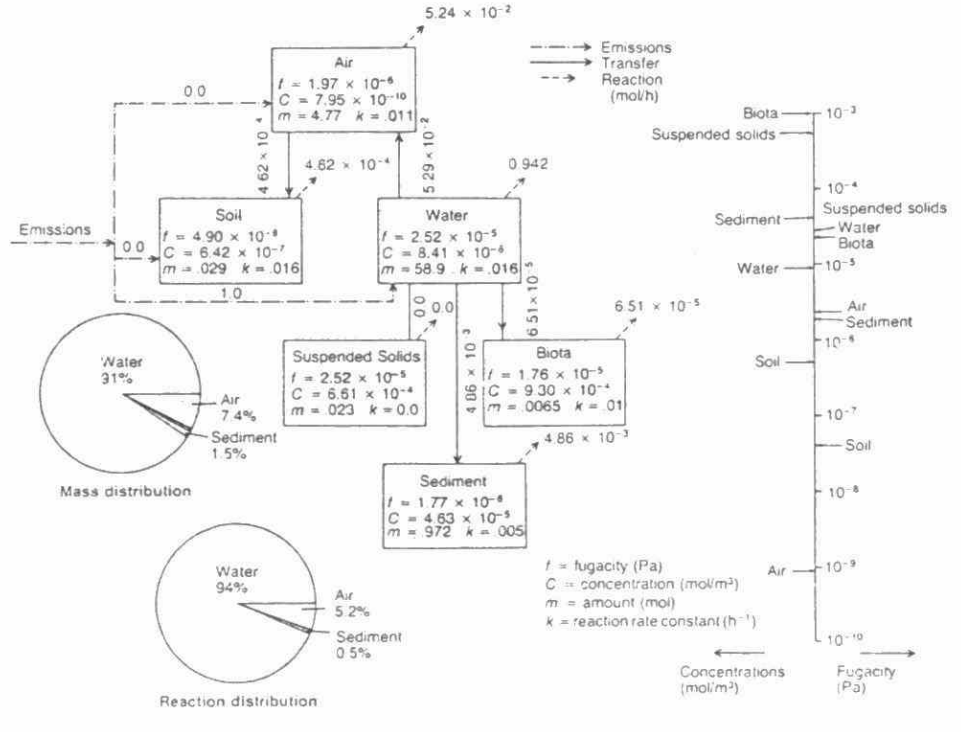


FIGURE 5c
Level III behavior profile with different compartment fugacities



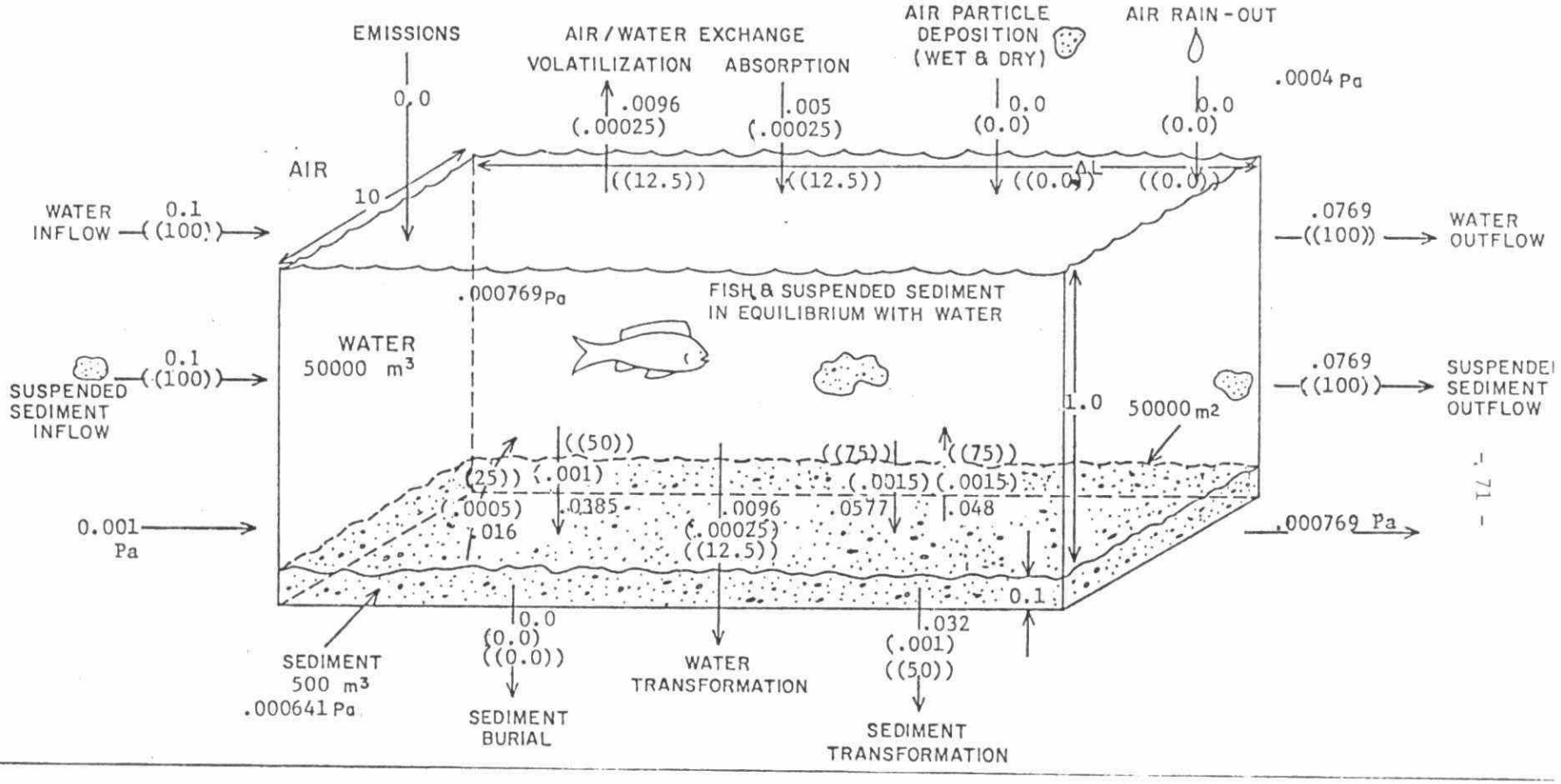


Figure 6. Mass balance over first river section for steady state solution. Mass fluxes are in mol/h. The quantities in single parentheses ( ) are B values, while those in double parentheses (( )) are D values. Fluxes are either the product of D and fugacity or B, fugacity and area, in this case 50000 m^2, i.e. W=10 and ΔL=5000.

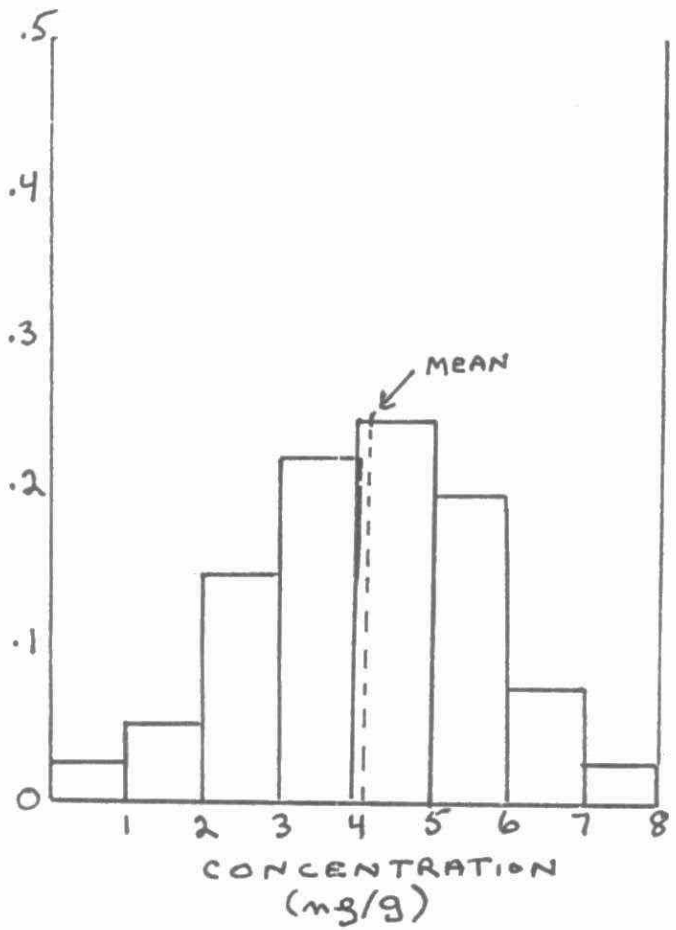


Figure 7a. Concentration distribution function

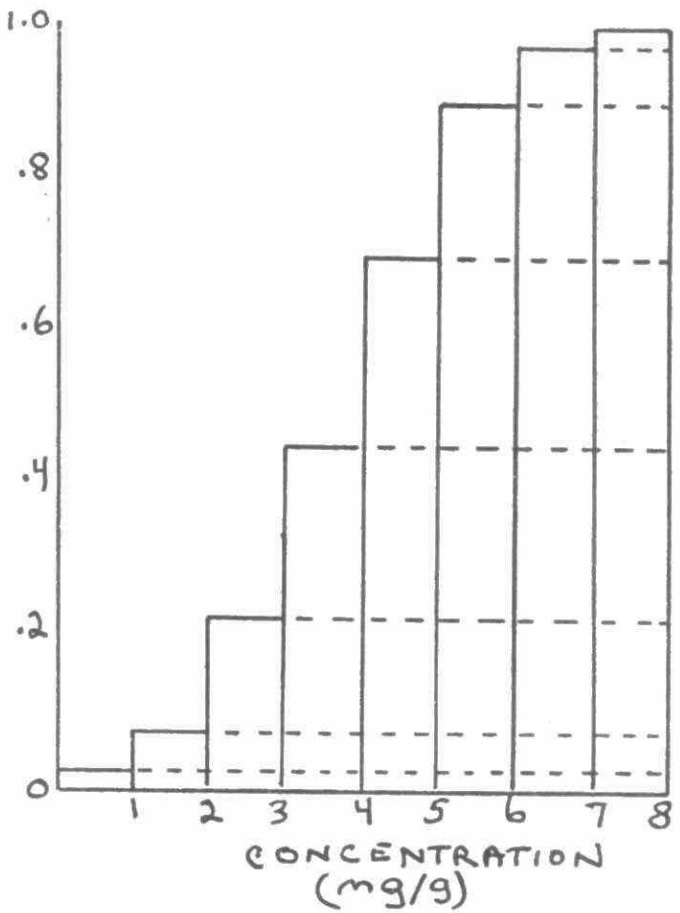


Figure 7b. Cumulative distribution function

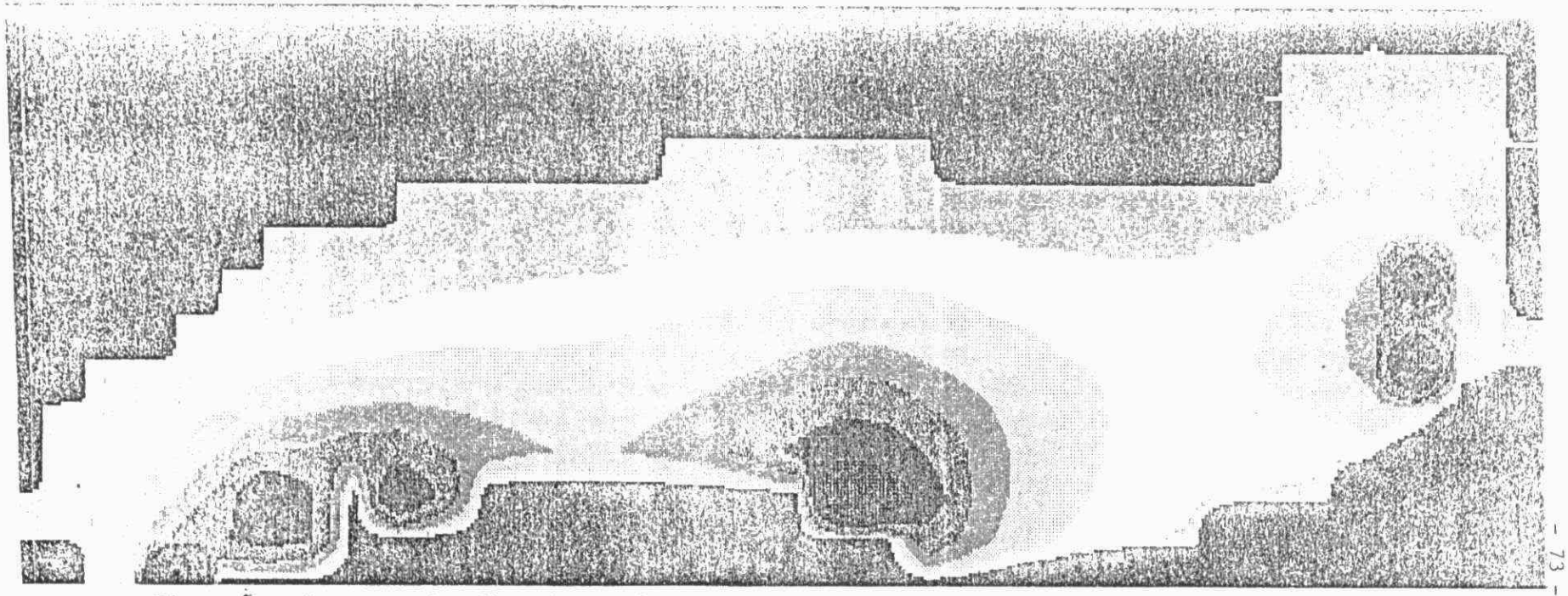


Figure 8a. Concentration distribution function

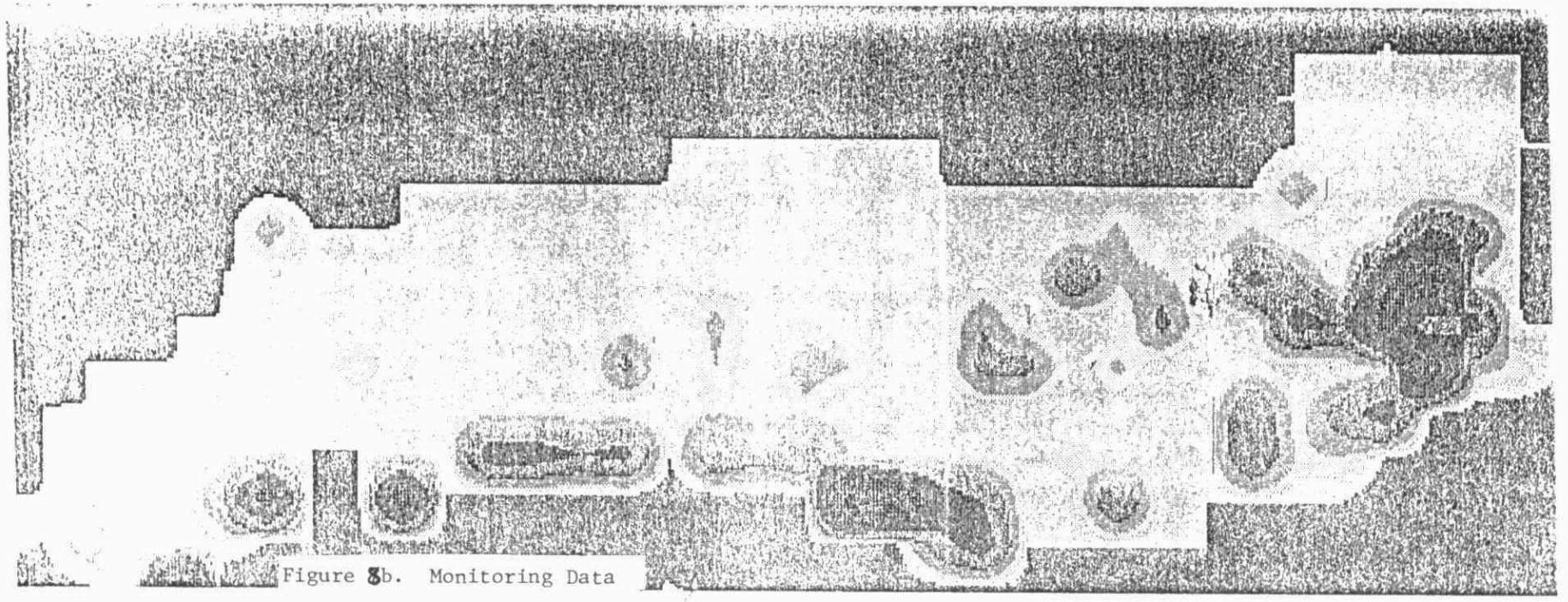


Figure 8b. Monitoring Data

ENVIRONMENTAL TOXICOLOGY

The toxic effects of chemicals can be classified in several ways and each method is useful for assessing the toxic impact of environmental chemicals. Administration of a toxic dose of a chemical and the subsequent examination of the adverse biologic and toxic effects can give a qualitative assessment of the toxicity of a chemical or chemical mixture. Dose-response studies are used to quantitate the adverse impacts of a chemical toxin and both qualitative and quantitative effects are important descriptions for any chemical.

A. TOXIC EFFECTS - QUALITATIVE ASSESSMENT

The qualitative assessments of toxic injury or adverse response can be classified in several possible ways.

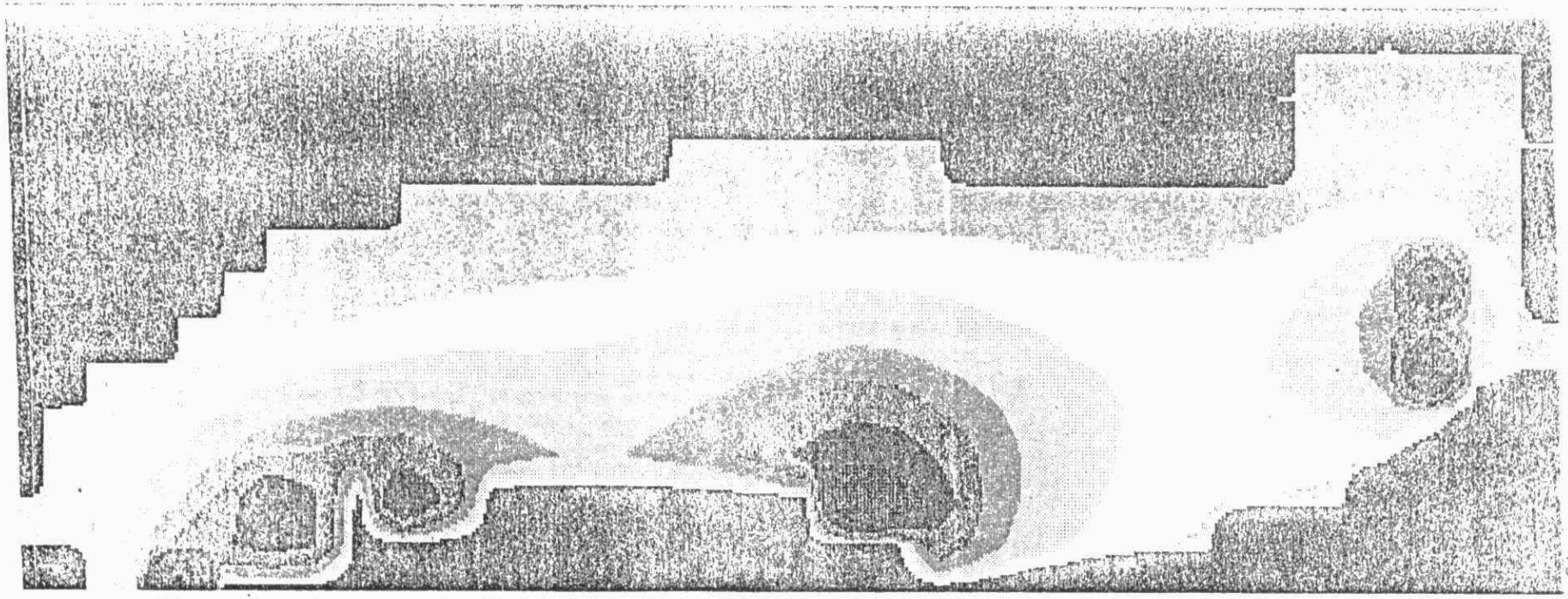
1. Type of Injury. The major types of adverse impacts include the following:

Neurotoxicity

Neurotoxicity refers to the delayed but persistent paralysis caused by certain organic phosphorus compounds. The classical example is "jake leg" paralysis caused by triorthocresyl phosphate. Most studies of neurotoxicity have attempted to learn which molecular configurations are capable of producing the phenomenon and which are not.

Teratogenesis

Although most research in teratogenesis has been centered on the nature of the phenomenon and the biologic factors wwich influence it, some quantitative studies have been reported (eg 2,3,7,8-TCDD). Teratogens are toxins which can be generally classified as agents which cause adverse effects in animal and human fetuses. Steep dosage-response curves for teratogenic action are not uncommon and, in fact, appear to be the rule. Many agents can be tolerated in low dosage without any recognizable effect on development or viability,



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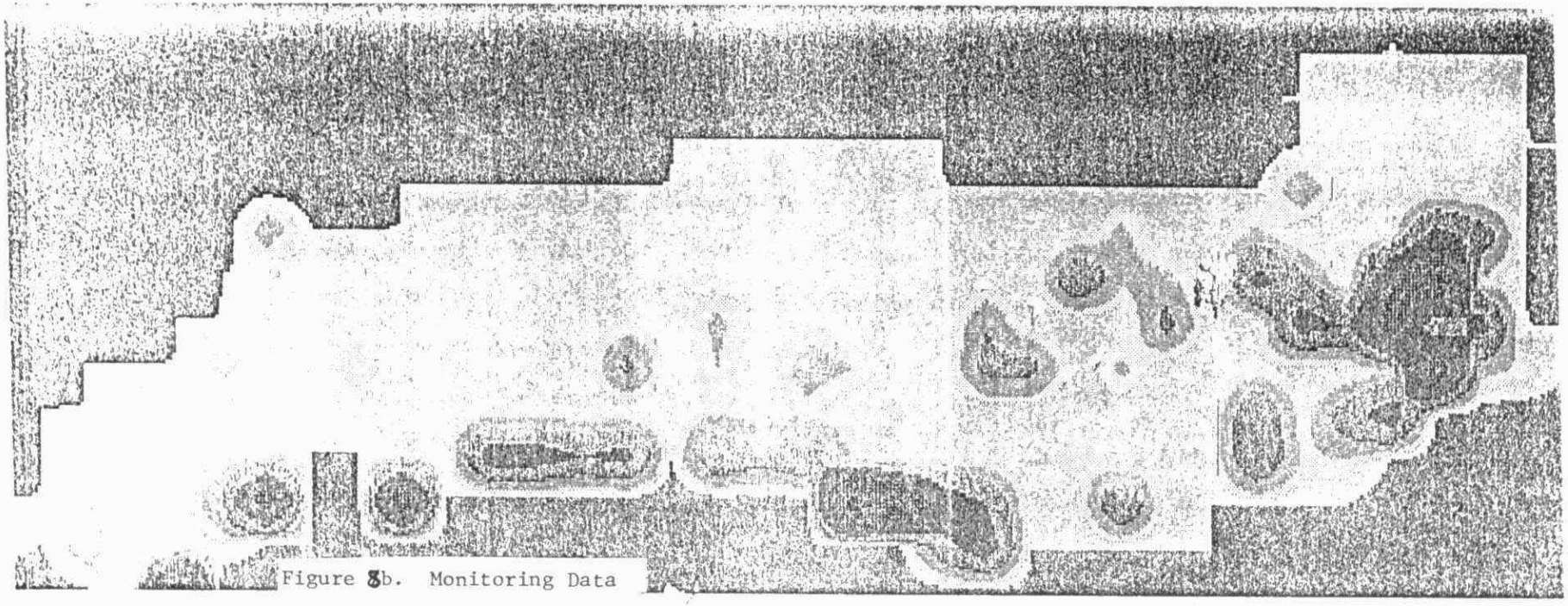

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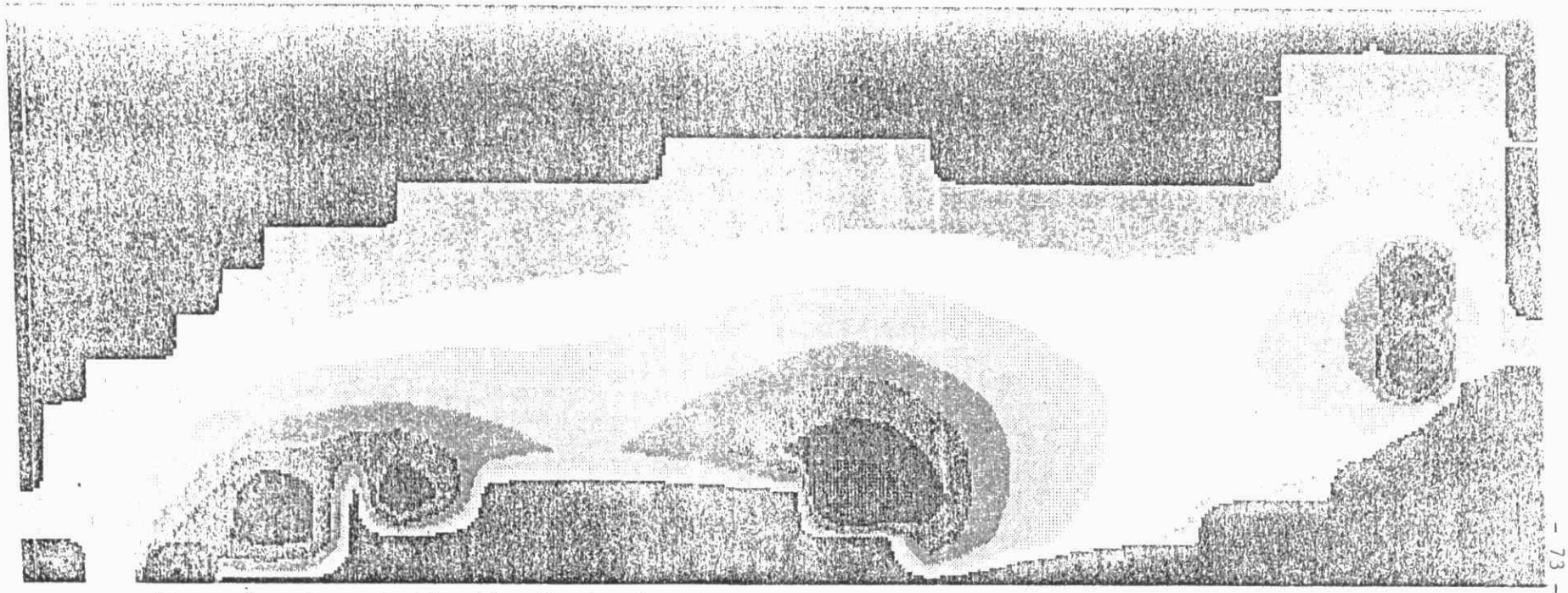

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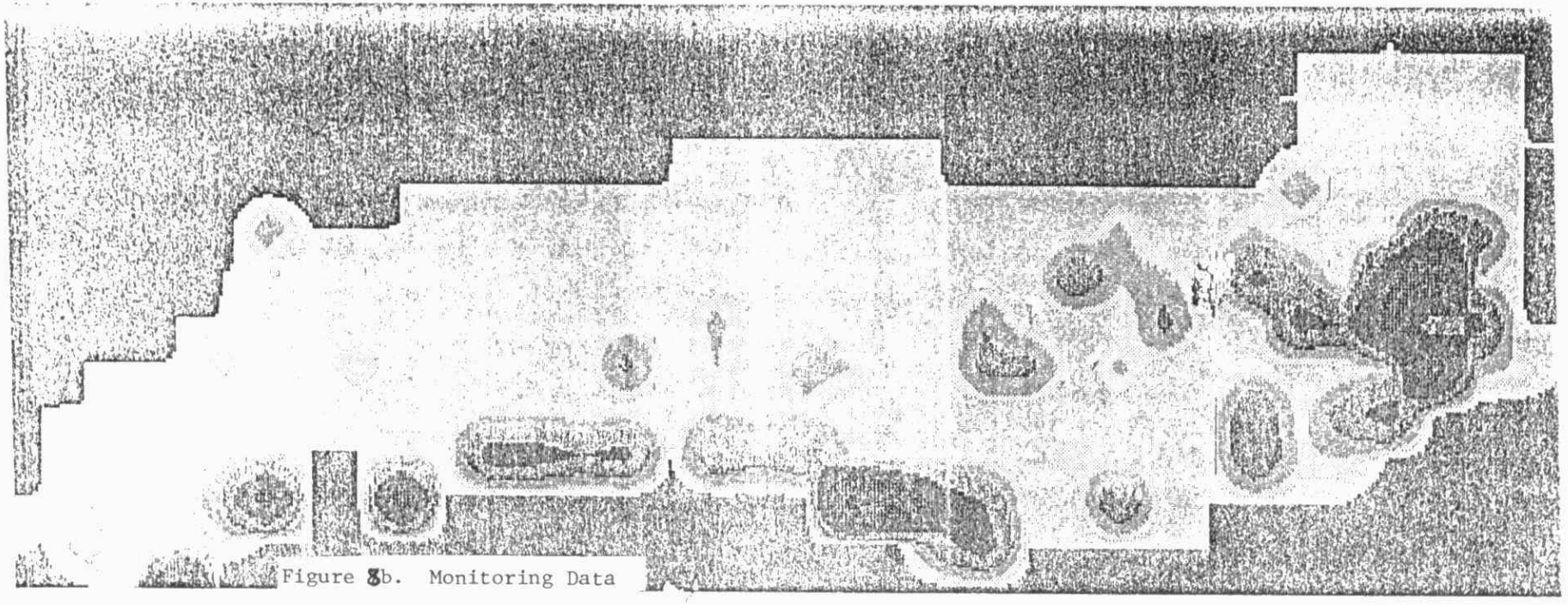


Figure 8b. Monitoring Data

ENVIRONMENTAL TOXICOLOGY

The toxic effects of chemicals can be classified in several ways and each method is useful for assessing the toxic impact of environmental chemicals. Administration of a toxic dose of a chemical and the subsequent examination of the adverse biologic and toxic effects can give a qualitative assessment of the toxicity of a chemical or chemical mixture. Dose-response studies are used to quantitate the adverse impacts of a chemical toxin and both qualitative and quantitative effects are important descriptions for any chemical.

A. TOXIC EFFECTS - QUALITATIVE ASSESSMENT

The qualitative assessments of toxic injury or adverse response can be classified in several possible ways.

1. Type of Injury. The major types of adverse impacts include the following:

Neurotoxicity

Neurotoxicity refers to the delayed but persistent paralysis caused by certain organic phosphorus compounds. The classical example is "jake leg" paralysis caused by triorthocresyl phosphate. Most studies of neurotoxicity have attempted to learn which molecular configurations are capable of producing the phenomenon and which are not.

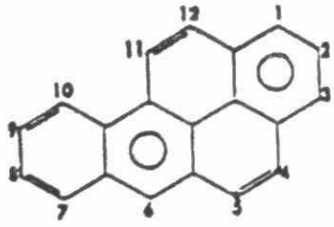
Teratogenesis

Although most research in teratogenesis has been centered on the nature of the phenomenon and the biologic factors wwich influence it, some quantitative studies have been reported (eg 2,3,7,8-TCDD). Teratogens are toxins which can be generally classified as agents which cause adverse effects in animal and human fetuses. Steep dosage-response curves for teratogenic action are not uncommon and, in fact, appear to be the rule. Many agents can be tolerated in low dosage without any recognizable effect on development or viability,

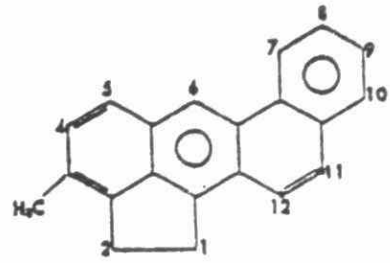
however, most teratogens become lethal to all embryos at higher dosages. Between these ranges of normality and lethality, there exists a narrow zone of dosages in which variable numbers of embryos survive with varying degrees of teratogenic involvement. A sharp rise of the dosage-reponse curve is also characteristic of the teratogenic action of x-radiation.

Carcinogenesis (Definition from Cassaret and Doull)

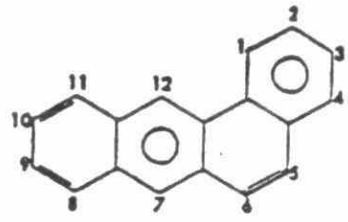
Chemical carcinogens are defined operationally by their ability to induce tumors. Four types of response have generally been accepted as evidence of tumorigenicity: (1) an increased incidence of the tumor types occurring in controls; (2) the occurrence of tumors earlier than in controls; (3) the development of types of tumor not seen in controls; and (4) an increased multiplicity of tumors in individual animals. Although the term "carcinogen" literally means giving rise to carcinomas, i.e., epithelial malignancies, in general usage it also includes agents producing sarcomas of mesenchymal origin. Furthermore, even benign tumors are included as evidence of carcinogenicity, a practice justified by the fact that no real carcinogen producing exclusively benign tumors has yet been identified. Chemicals capable of eliciting one of these tumorigenic responses, and which are thereby classified as carcinogens, comprise a highly diverse collection of chemicals, including organic and inorganic chemicals, solid-state materials, hormones, and immunosuppressants. For some of these chemicals, such as tumor enhancers or promoters, the designation "carcinogen" is perhaps unfortunate, but inescapable, since these chemials in specific situations do increase the yield of spontaneously occurring cancers. In order to draw attention to the differences in properties of the diverse agents that can be considered as carcinogens, we have developed a classification based on the mode of action of carcinogens. This classification is important not only for theoretic, fundamental reasons, but also because different types and classes of carcinogens require distinct



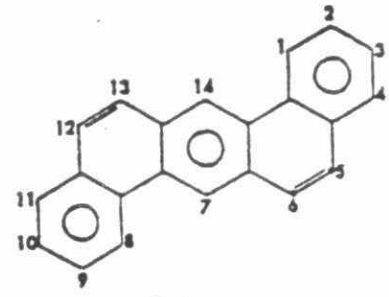
Benzo[a]pyrene



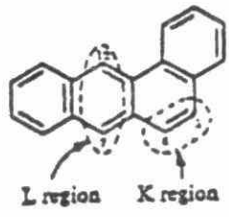
3-Methylcholanthrene

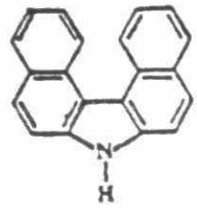


Benz[a]anthracene

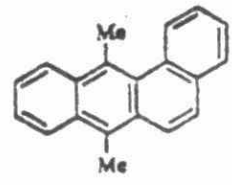


Dibenz[a,h]anthracene





Dibenz[c,g]carbazole



7,12-Dimethylbenz[a]anthracene

Some polycyclic aromatic carcinogens

Figure 1

however, most teratogens become lethal to all embryos at higher dosages. Between these ranges of normality and lethality, there exists a narrow zone of dosages in which variable numbers of embryos survive with varying degrees of teratogenic involvement. A sharp rise of the dosage-reponse curve is also characteristic of the teratogenic action of x-radiation.

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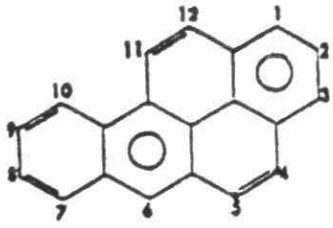
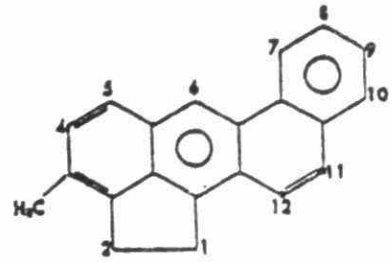
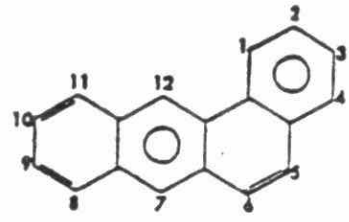


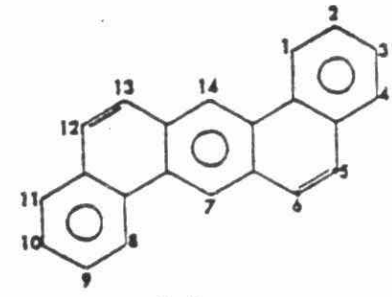
Benzo[a]pyrene



3-Methylcholanthrene



Benz[a]anthracene



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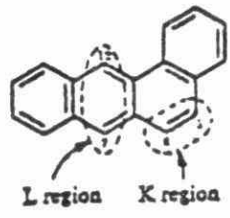
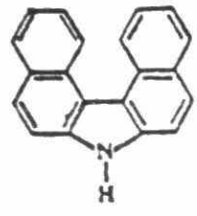
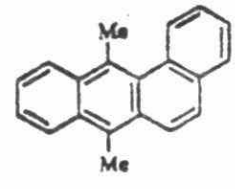




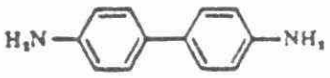
Dibenz[c,g]carbazole



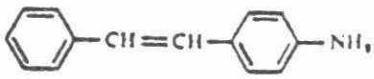
7,12-Dimethylbenz[a]anthracene

Some polycyclic aromatic carcinogens

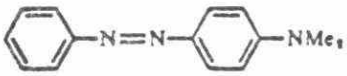
Figure 1



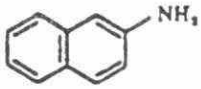
Benzidine



4-Aminostilbene

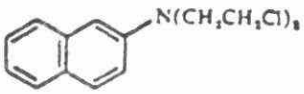


4-Dimethylaminoazobenzene

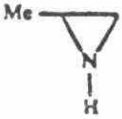


β-Naphthylamine

Somecarcinogenic amines and azo-compounds



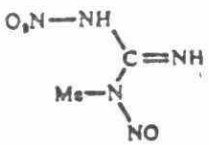
Chlornaphazin



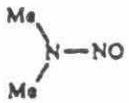
Propyleneimine

β-Propiolactone

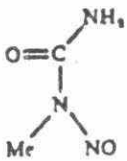
Some carcinogenic alkylating agents



N-Methyl-*N*-nitrosonitroguanidine



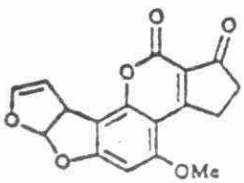
N-Nitrosodimethylamine



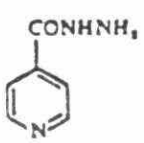
N-Methyl-*N*-nitrosourea

N-Nitrosopiperidine

Some carcinogenic N-nitroso-compounds



Aflatoxin B_1



Isoniazid

Vinyl chloride

Nickel carbonyl

Miscellaneous carcinogens

Figure 1 (cont.)

regulatory actions to ensure safety. The following diagrams illustrate the structures of several carcinogens and major research efforts are devoted to understanding how chemicals with such diverse structures can elicit similar carcinogenic effects.

Mutagenesis

Like carcinogenesis, mutagenesis describes a process in which genes (cellular DNA) undergo a specific type of damage, namely point or gene-locus mutations. The result of this type of damage can result in a host of adverse human health impacts. Professor Bruce Ames at Berkeley has utilized several bacterial strains of Salmonella typhimurium which are highly susceptible to mutagens, as a short term in vitro assay for detecting carcinogens (as mutagens). Several mutagenic assays can be used in this way as "prescreens" for potential cancer-causing chemicals.

Allergic Reactions (from Cassaret and Doull)

Chemical allergy is an adverse reaction to a chemical resulting from previous sensitization to that chemical or to a structurally similar one. The term "hypersensitivity" has often been used to describe this allergic site but should be avoided because the term is more appropriately used to describe the response of the subjects at the lower end of the frequency distribution in a quantal dose-response curve. Thus, allergic reaction or sensitization reaction, not hypersensitization reaction, should be used to describe the situation where a preexposure of the chemical is required to produce the toxic effect via an antibody.

An allergic reaction does not usually exhibit a typical sigmoid dose-response curve as observed for most toxic responses. Because of this apparent lack of a dose-response, some people have not considered the allergic reaction to be a toxic response. However, since the allergic response is an undesirable, adverse, deleterious effect, it should be regarded as a toxic

response. Toxicity is defined as the inherent ability of a chemical to adversely affect living organisms. Sensitization reactions are often very severe and many are fatal.

Metabolism, Storage and Enzyme Induction

Exposure of any biological system to a chemical results in an immedite response by the host to handle this chemical. These processes involve absorption, distribution, metabolism and ultimately excretion of the xenobiotic (see Figure) and the rates of these processes are dependent on the host and the structure of the chemical. In addition, many toxic chemicals induce (or inhibit) specific enzymes and this biologic effect can ultimately play an important role in mediating a toxic response.

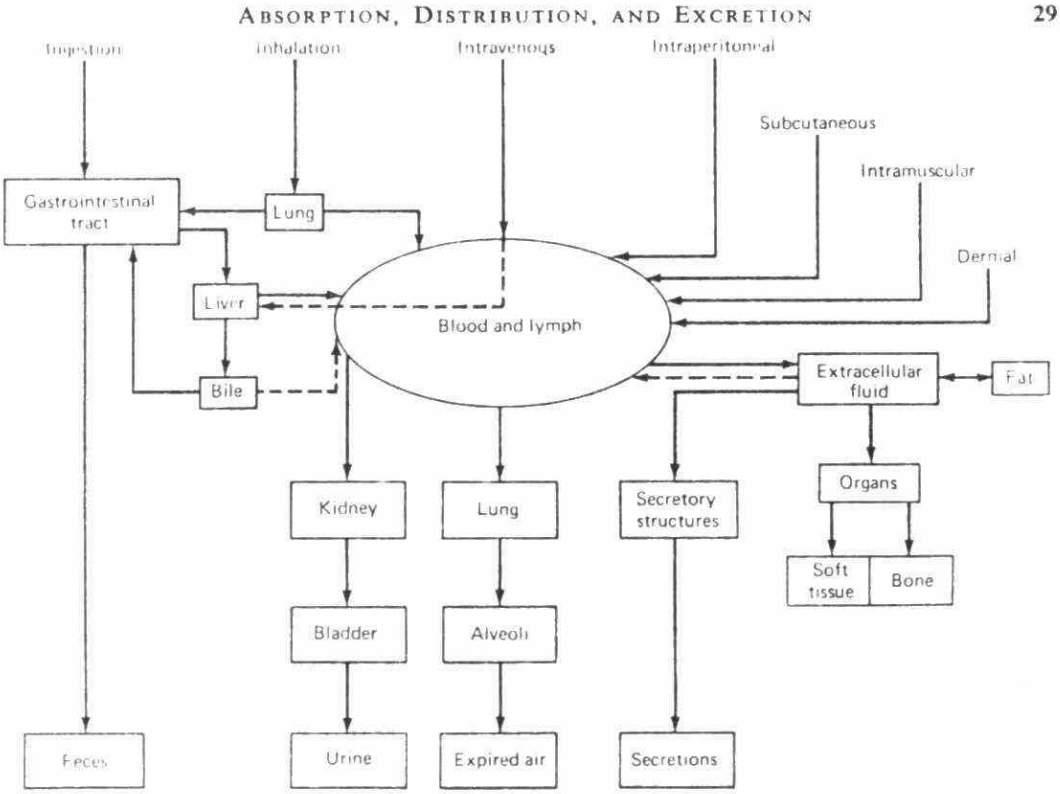


Figure 3–1. Routes of absorption, distribution, and excretion of toxicants in the body. (Courtesy of L. J. Casarett.)

2. Immediate versus Delayed Toxicity (from Cassaret and Doull). Immediate toxicologic effects can be defined as those that occur or develop rapidly after a single administration of a substance, while delayed effects are those that occur after the lapse of some time. Carcinogenic effects of chemicals usually have a long latency period, often 20 to 30 years, before tumors are observed. For example, the vaginal and uterine cancer produce by diethylstilbestrol in young women was due to their in utero exposure to diethylstilbestrol taken by their mothers to prevent miscarriages. Also, delayed neurotoxicity is observed after some organophosphorus anticholinesterase agents. The most notorious of the compounds that produce this type of neurotoxicity effect is triorthocresylphosphate (TOCP). The effect is not observed until at least several days after exposure to the toxic compound. On the other hand, one can find many substances, such as short-acting barbiturates, that yield immediate toxic effects on each of a series of exposures but fail to produce delayed effects.

3. Reversible and Irreviersible Effects. Toxic injury by chemicals can also be classified according to their reversibility or irreversibility. some chemicals such as CCl_4 or $CHCl_3$ are hepatotoxins and their effects at low doses are reversible and their damage is repaired. However, at sufficiently high dose levels these effects are irreversible and can result in death. Carcinogens are classical examples of toxins which cause irreversible damage.

4. Local Versus Systemic Effects (from Casaret and Doull). Another distinction between types of effects is made on the general locus of action. Local effects refer to those that occur at the site of first contact between the biologic system and the toxicant. Examples of local effects are demonstrated by the ingestion of caustic substances or inhalation of irritant materials. The reverse of local effects are systemic effects that require

absorption and distribution of the toxicant to a site distant from its entry point at which point effects are produced. Most substances, except highly reactive materials, produce systemic effects. For some materials, both effects can be noted. For example, tetraethyl lead produces effects on the skin at the site of absorption and then is transported systemically to produce its typical effects on the central nervous system and other systems. If the local effect is marked, as with a severe acid burn, there may also be indirect systemic effects, in this example, damage to the kidney even though the substance has not reached the kidney.

Most chemicals that produce systemic toxicity do not cause a similar degree of toxicity in all organs but usually produce the major toxicity to one or two organs. These are referred to as the target organs of toxicity for that chemical. The target organ of toxicity often is not the site of concentration of the chemical. For example, lead is concentrated in bone but its toxicity is due to lead in soft tissues. Likewise, DDT is concentrated in adipose tissue but produces no known toxic effects there.

FACTORS WHICH EFFECT TOXICITY (from Cassaret and Doull)

It is convenient to divide the factors that influence toxicity into those that are related to (1) the poison or toxic agent, (2) the exposure situation, and (3) the subject. The factors that are related to the subject can be further divided on the basis of whether they are internal (inherent in the subject) or external to the subject (environmental). This general classification has been used in preparing the listing of toxicity-influencing factors shown in Table 5-1.

Much of the controversy that is associated with the interpretation of the results of supposedly comparable toxicologic investigations is the result of a failure to receognize the importance of these factors and the ability of even

slight changes in the experimental protocol to alter markedly the response of the test system. Toxicologists need to keep these factors in mind in carrying out their predictive resopnsibilities (safety evaluation) and also in their activities related to detection (forensic toxicology) and to the diagnosis and treatment of poisoning (clinical toxicology).

Table 5-1. A CLASSIFICATION OF TOXICITY-INFLUENCING FACTORS

1. *Factors Related to the Toxic Agent*
   - Chemical composition (pH, choice of anion, etc.)
   - Physical characteristics (particle size, method of formulation, etc.)
   - Presence of impurities or contaminants
   - Stability and storage characteristics of the toxic agent
   - Solubility of the toxic agent in biologic fluids
   - Choice of the vehicle
   - Presence of excipients: adjuvants, emulsifiers, surfactants, binding agents, coating agents, coloring agents, flavoring agents, preservatives, antioxidants, and other intentional and non-intentional additives

2. *Factors Related to the Exposure Situation*
   - Dose, concentration, and volume of administration
   - Route, rate, and site of administration
   - Duration and frequency of exposure
   - Time of administration (time of day, season of the year, etc.)

3. *Inherent Factors Related to the Subject*
   - Species and strain (taxonomic classification)
   - Genetic status (littermate, siblings, multigeneration effects, etc.)
   - Immunologic status
   - Nutritional status (diet factors, state of hydration, etc.)
   - Hormonal status (pregnancy, etc.)
   - Age, sex, body weight, and maturity
   - Central nervous system status (activity, crowding, handling, presence of other species, etc.)
   - Presence of disease or specific organ pathology

4. *Environmental Factors Related to the Subject*
   - Temperature and humidity
   - Barometric pressure (hyper- and hypobaric effects)
   - Ambient atmospheric composition
   - Light and other forms of radiation
   - Housing and caging effects
   - Noise and other geographic influences
   - Social factors
   - Chemical factors

B. TOXIC EFFECTS - QUANTITATIVE ASSESSMENT

Scientific study of the effects of chemical or physial agents on living organisms requires measurement. A distinction is made as to whether each measurement involves an agent alone (dose) or an agent in relation to an organism (dosage). A 1.0-mg dose of a compound is identical no matter whether it is administered to a 20-g mouse or a 5,000-kg elephant but the dosages are vastly different, being 50 mg/kg for the mouse and 0.002 mg/kg for the elephant. The susceptibility of different species or even different individuals can be compared precisely only if their body weight is taken into account. Thus, if dose levels can be considered in terms of body weight (e.g. weight of chemical/body weight or moles of chemical/body weight) several types of quantitative assessments of toxic response can be made.

1) $\underline{LD_{50} \text{ or } ED_{50}}$ The ED_{50} is defined as the dose of a chemical which causes an effect in 50% of the test animals. The LD_{50} is an ED_{50} in which the effect measured is death. The two Tables shown below give examples of the LD_{50} values for several chemicals and summarize an LD_{50} rating chart (from Cassaret and Doull). It should be noted that the ED_{50} values obtained for any toxic

Table 2-1. APPROXIMATE ACUTE LD50s OF A SELECTED VARIETY OF CHEMICAL AGENTS

AGENT	LD50 (mg/kg)
Ethyl alcohol	10,000
Sodium chloride	4,000
Ferrous sulfate	1,500
Morphine sulfate	900
Phenobarbital sodium	150
DDT	100
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
d-Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.10
Dioxin (TCDD)	0.001
Botulinus toxin	0.00001

Table 2-2. TOXICITY RATING CHART

	PROBABLE ORAL LETHAL DOSE FOR HUMANS	
TOXICITY RATING OR CLASS	DOSE	FOR AVERAGE ADULT
1. Practically nontoxic	> 15 g/kg	More than 1 quart
2. Slightly toxic	5–15 g/kg	Between pint and quart
3. Moderately toxic	0.5–5 g/kg	Between ounce and pint
4. Very toxic	50–500 mg/kg	Between teaspoonful and ounce
5. Extremely toxic	5–50 mg/kg	Between 7 drops and teaspoonful
6. Supertoxic	< 5 mg/kg	A taste (less than 7 drops)

agent is subject to all the factors which can effect the magnitude of the response. The following Figure summarizes a classical warfarin (rat poison) LD_{50} study. A single 1-dose test gives an LD_{50} of 1.6 mg/kg with a survival time of 5-10 days; whereas the LD_{50} for a multiple dose 90-day feeding study was 0.077 mg/kg with a survival time of 3-34 days.

2. $\underline{EC_{50} \text{ or } LC_{50}}$ (from Hayes). An EC_{50} is a statistical estimate of the concentration of a toxicant in the ambient medium necessary to produce a particular effect in 50% of a very large population under specified conditions. An LC_{50} is a special case of the EC_{50} in which the effect recorded is death. EC_{50} and LC_{50} values are determined in a similar way to ED_{50} and LD_{50} values, ie., probits representing the percentages of animals showing a response in a series of tests are related to the logarithms of the concentrations that produced the responses.

3. ET_{50} or LT_{50} (from Hayes). An ET_{50} is a statistical estimate of the interval or time from dosage to a specific all-or-none response of 50% of the organisms in a very large population subjected to a toxicant under specified conditions. As used here, an all-or-none effect may be a specified level of a quantitative response, for example, time of appearance of first tumor or time at which the systolic blood pressure reaches 150 mm Hg. An LT_{50} is a special case of an ET_{50} in which the effect reported is death.

4. Regulatory or Predictive Toxicology. (in part from Cassaret and Doull). The main purpose of toxicity tests is to provide a data base that can be used to assess the risk (or evaluate the hazard) associated with a situation in which the chemical agent, the subjet, and the exposure conditions are defined. It is evident that the ideal situation is one in which the agent, subject, and exposure conditions used for the toxicity tests are identical to those which will be encountered in the situation where the risk assessment is desired. Thus, by carrying out toxicity studies on various forms of birds, fish, and other wildlife, it is usually possible to establish with reasonable certainty a safe or no-observed-effect level (NOEL) for the species tested [also referred to as no-effect level (NEL) and no-observed-adverse-effect level (NOAEL)]. In most cases, however, there are differences between the toxicity testing situation and the "real world" situation where the risk estimate is desired that require extrapolation of the data base or prediction of the hazard in areas not covered by the data base. Changes in the formulation of the agent, the use of animal tests for estimating risk to man, changes in route, dose, duration, and frequency of exposure are all examples of situations in which predictive toxicology is employed. In most cases, predictive toxicology provides the risk portion of the risk-benefit analysis, which is the basis of regulatory toxicology.

Having determined risk factors this quantitative data can be used to promulgate regulatory guidelines for exposure. These guidelines can be set for regulating a) workplace exposure to industrial toxins, b) levels of pesticides, food additives and other xenobiotics permitted in foods, and c) levels of chemicals which are discharged into the environment. These regulatory acronyms (ADI - acceptable daily intake; TLV-, threshold limit value etc) can be determined using the following approach, eg

$$\text{Permissable Exposure Level} = \frac{\text{NEL (sensitive species)}}{\text{Safety Factor}}$$

It is readily apparent that there are several inherent problems in the regulatory level-setting process, for example, a) was the NEL determined on the most sensitive species, b) was the most sensitive effect measured, c) how do we know that humans (or the potential host) are not more sensitive than the test animal species, d) are interactive effects with other chemicals accounted for, and e) is the safety factor high enough. These are challenging questions for toxicologists and regulators and it is apparent that both groups must be aware of the inherent problems which must be solved to arrive at an acceptable answer to these questions.

REVIEW ARTICLE

Chemical Carcinogenesis

A Biologic Perspective

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DEVELOPMENTS in the past two decades or so have led to a progressive shift in emphasis from the individual to the environment in the perception of the causation and pathogenesis of cancer. This change is bringing into clearer focus both the genetic makeup as a risk determinant for the effectiveness of chemicals, radiation, or viruses as causes of cancer and the distribution and properties of the possible chemicals, radiation, and viruses themselves. Both aspects, the environment and the risk components, are now beginning to receive increasing attention in the prevention of cancer. Although many factors have contributed to this radical shift in perception, two of the most important are the general acceptance of cigarette smoking as a major factor in the increasing incidence of lung cancer and the multiplication in the number of examples of radical shifts in organ or tissue distribution of primary cancers with the migration of distinctive ethnic groups of individuals from one geographic location to another.[1,2]

The available evidence indicates the likelihood of several, if not many, components in this environmental outlook. Diet, carcinogenic chemicals, radiation, and viruses are among the known major factors that appear to be involved. The largest group by far are chemicals, man-made and naturally occurring, in the workplace, in the home, and in the medical therapeutic armamentarium. More than thirty chemicals or mixtures are known to be involved in the genesis of cancer in human beings.[3] In addition, an increasing number of dietary and other environmental components, including pesticides, fungicides, and other xenobiotics widely used in food production, are being recognized as agents that can either promote or diminish the development of cancer.

The number and diversity of such agents warrant a greater interest in chemical carcinogenesis by physicians, medical scientists, and other health-related professionals. This increasing awareness has naturally led to the concept that cancer can be largely prevented by concentration of virtually all our efforts in the prevention of cancer on the identification, by appropriate short-term assay procedures, of potential carcinogenic chemicals and their elimination from our environment.

This is, without doubt, a laudable and desirable goal and is feasible in isolated instances. However, it is already evident that such an undertaking *in toto* would involve radical changes in our way of life without any guarantee that the new patterns of living would not simply shift the spectrum of the observed neoplasms without major changes in overall cancer incidence. Some evidence does exist that groups of individuals, such as Mormons, Seventh Day Adventists, and others, who practice a much more ascetic life style with much less exposure to tobacco and alcohol, and sometimes with different dietary and sexual patterns, have low incidences of some types of cancer.[4] However, even among such lower-incidence groups, many cancers have incidences equal to or

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higher than appropriate groups of controls. Although the overall incidence of cancer is lower in such select groups than in the population generally, cancer is still a major cause of death.

Thus, from the pragmatic viewpoint, it would seem reasonable for us to foster a much greater interest in how cancer develops, in order to devise innovative ways to interrupt the process. In addition, it is becoming increasingly apparent that any study of end-stage cancers *per se* is fraught with so many variables and pitfalls as to frustrate any truly scientific analysis of the essential nature of malignant neoplasia at this time. A historic approach, emphasizing pathogenesis, appears to offer a much more rational and feasible approach, providing suitable models for cancer development can be discovered.

In this review, emphasis will be given to mechanisms, not phenomena. It is the conviction of the author that an analysis of how cancer develops with chemicals, that is, the pathogenesis of neoplasia, with an integration between the molecular and biochemical levels of organization on the one hand and the cellular and tissue biologic levels on the other, offers major opportunities to generate important new insights into the genesis and nature of neoplasia.

Multistep Nature of Carcinogenesis

It is now appreciated that cancer development in human beings in various sites takes many years and that during the so-called latent period, progressive tissue and cellular changes can be seen. Often, new cell populations appear that probably represent stages or steps in the cellular evolution from normal, through initiated, preneoplastic and premalignant cells to highly malignant neoplasia.[5-7] The research in experimental animals has concentrated heavily upon the early events and has suggested the existence of common patterns of early preneoplastic changes in several organ systems and in several species.[7] Research in humans has concentrated largely upon the later events and has confirmed the occurrence of atypical hyperplasias, dysplasia, and carcinoma *in situ* as probable precancerous steps in several organs later in the process.[7,8] However, the critical properties of each of the different cell populations that relate to their possible roles in cancer development and the manner in which the two aspects of carcinogenesis, the experimental and the human, fit together into some biologically meaningful pattern or patterns remain as challenges.[7]

The conceptual advances made in the late 1930s and 1940s concerning the first two early steps in chemical carcinogenesis, *initiation* and *promotion*, are well known in general.[5-7,9] However, their mechanistic bases are only now becoming slowly unfolded, and these will be major topics for review. Their basic validity for human cancer development has been established in a few instances and as such is reassuring both for the physician and for the scientist. For example, a relatively brief exposure to diethylstilbestrol (DES) during pregnancy may be associated with the development of vaginal neoplasia some 15 to 25 years later in the daughters of those to whom it was administered.[10] Parenthetically, it should be mentioned that it is not yet known whether the DES is acting in this situation as a chemical carcinogen or whether the effect is predominantly hormonal. Again, a limited exposure for only a few months to a known chemical hazard, such as vinyl chloride, may lead many years later to the appearance of angiosarcoma of the liver.[11] Nonneoplastic cellular and tissue changes are seen during the apparent latent period.

Agents and Processes in the Analysis of Carcinogenesis

Given the increasing success that has been achieved in the identification and characterization of cancer-inducing chemicals beginning in the 1920s, the study of chemical carcinogenesis has been dominated to a large and expanding degree by the principle that the understanding of the chemical and physical properties of chemical carcinogens and of their immediate interactions with target and other tissues will lead to the elucidation of carcinogenic processes. With the discovery of promoting agents and the clarification of the chemistry of some of the materials most active for skin, such as croton oil, this reductionist approach concentrating on agents was extended to postinitiation phenomena. No one who is familiar with the successes in the areas of chemistry and biochemistry of initiators and promoters can take exception to this scientific approach *as a first step*. However, the increasing study of the *responses of living cells and tissues* to a wide variety of toxic or potentially toxic agents at both the biochemical and the biologic levels of organization over the past few decades has made it eminently evident that the living organism is by no means a passive object for change. On the contrary, it is a very dynamic partner in any interaction between agent and target tissue. In fact, as emphasized a few years ago, a cell is "the smallest *integrating* unit in biology: a pseudo-intelligent computer that receives, screens, changes, reacts to and adapts to a host of environmental signals, all of this activity apparently designed, through evolution, for cell survival and host survival."[12] In other words, it is un-

likely that we will be very successful in developing a rational understanding of cancer induction by concentrating our focus on the agents alone. An increasing interest and study of the *different processes* that constitute carcinogenesis, *as processes*, would seem to be a prerequisite for elucidation of its essential nature. Although investigators pay passing lip service to this approach in chemical carcinogenesis, relatively little scientific attention is devoted to this type of study. This narrow approach to the study of toxic responses, ie, to the "toxic components in toxicology," is not unique to research workers in chemical carcinogenesis but remains the dominant scientific philosophy in toxicology generally.

There are at least two practical consequences of this simplistic view of chemical carcinogenesis. The first, as already mentioned, is the naive conviction that "cleaning up our environment" is all that will be necessary to control the bulk of cancer in the population. Although the removal of identifiable hazards, such as those generated during cigarette smoking or in the workshop, must be of high priority in any battle with cancer, there is no proof that some of the major forms of cancer in any geographic location (eg, cancers of the colon, breast, and uterus in North America), lend themselves to this approach. Also, in the case of promoters that usually require prolonged exposure, it is questionable whether the "environmental approach" is adequate to cope with the problem effectively.

The second concerns the scientific attitude to research in chemical carcinogenesis. It has been pointed out sporadically that the response patterns of living tissues to carcinogens are by no means unique to this group of toxic agents. Rather, a view of the carcinogenic process from a broader perspective of evolution and adaptation generally seems more reasonable.[7,13-15] Since the tags we place on chemicals are not a recognizable medium of communication at the cellular level, are we narrowing our perspective too much by the literal use of terms such as "carcinogen"? It is very likely that most, if not all, the changes induced in target cells by "carcinogens" have no direct relevance to cancer but are a reflection of more general response patterns to injury at the molecular as well as cellular level. In this context, it is critical to know whether the changes in cells seen on exposure to known chemical carcinogens have any direct relevance to the behavior of an ultimate population of malignant neoplastic cells or whether such changes at each step simply increase the probability for the occurrence of subsequent steps that progressively move toward cancer and that have a greater mechanistic relationship to malignancy as they evolve. If this latter view has any validity and usefulness, do these different cell populations, especially the early ones, play a functional role in the adaptation of the organism to a hazardous environment[7]?

It is noteworthy that there is a major discrepancy between the number and apparent diversity of immediate *chemical and biochemical* effects of different carcinogens, promoters, and other modulators of carcinogenesis, such as vitamin A analogs, and the number of immediate *biologic* end-points of the exposure to such agents. For example, chemical carcinogens induce a wide array of discrete chemical changes in the DNA in target cells,[16] yet the first observable biologic end-points, a nodule, a papilloma, a polyp, are virtually indistinguishable when induced by quite different carcinogens. The same is true in principle for later focal lesions and for the cancers that ultimately appear. In other words, the response patterns to carcinogens (and to other potentially toxic agents) are often very similar, if not identical, in appearance and behavior, even when induced by agents of widely divergent chemical and physical properties. This similarity indicates the existence of a sort of biologic direction to the cellular response to many toxic agents, including carcinogens. Thus, a large, diverse array of agents added to a system or organism often generates a common pattern of response. This lack of correspondence between *diversity of input* and *commonality of output* poses a major problem for interpretation in a complex process such as carcinogenesis and emphasizes the necessity for the development of models in which one can measure and relate input and output for each step. In at least two well-defined systems for chemical carcinogenesis, the skin and the liver, such a close correlation is becoming feasible[7] and should be encouraged.

Initiators and Initiation

Cancer development with chemicals can begin with a brief exposure (a few hours or days) to an activated form of a carcinogen, and the chemical need not be present as such ever again, as far as can be determined. This point is valid not only with regard to the skin, which was the first tissue in which it was demonstrated clearly, but also in liver, colon, mammary gland, urinary bladder, kidney, brain, and other organs or tissues.[6,7,17,18] The brief exposure to the chemical induces neither a cancer cell nor a neoplastic cell, but rather some cell that can be differentially stimulated to produce a focal proliferation. By "neoplastic cell" is meant a cell that can proliferate without the need for an added or known stimulus for

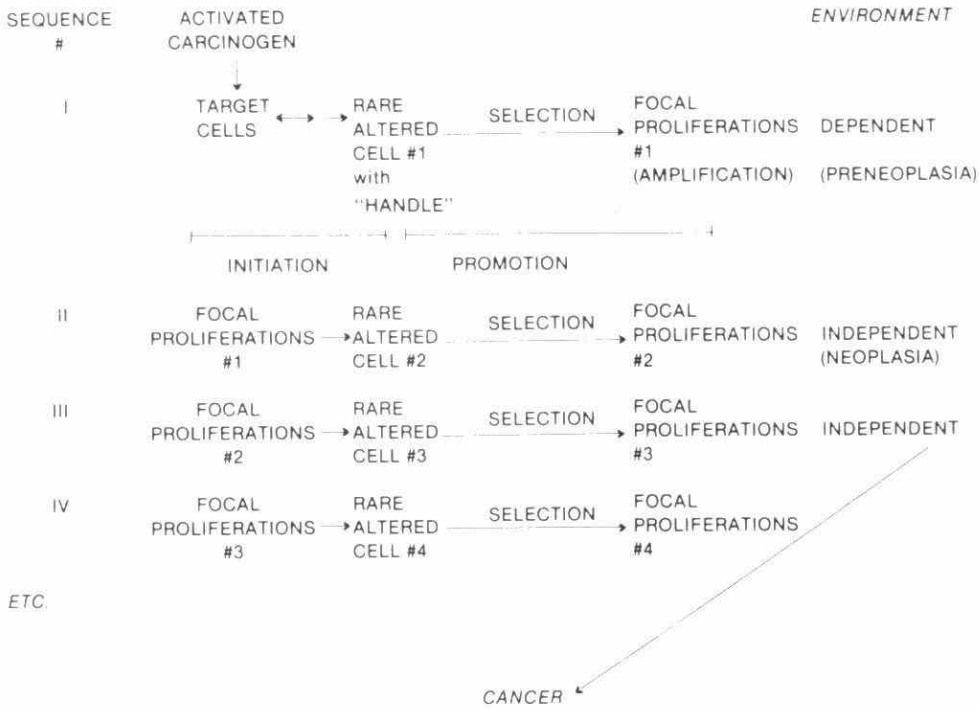

Figure 1—Proposed sequences of rare events-selection (promotion) initiated by exposure to an appropriate dose of a carcinogen coupled with cell proliferation. The number of sequences leading to cancer is unknown. The first sequence (initiation, promotion) is environment-dependent. All subsequent sequences would appear to be self-generating.

growth, ie, a cell that has acquired some degree of autonomy.

The existence of an initiated state cannot be detected *per se* but makes itself evident only when some promoting or selecting environment is created, ie, by the occurrence of subsequent focal proliferation. Thus, the concept of initiation is intricately bound up with the subsequent appearance of discrete proliferative lesions.

The separation of this first sequence, initiation-promotion, is justified because of the dependence of each of its steps on the environment for their appearance (Figure 1). With a single exposure to an initiating dose of a carcinogen, the further history is closely dependent upon the imposition of a suitable promoting environment. This environment can be created by a "pure" promoter or by further exposure to a carcinogen. In the former case, repeated exposure over a relatively long period (weeks to months in rats or mice) is usually required, In the latter case, a more brief exposure to an active carcinogen may suffice.

This first sequence generates a new cell population that is at risk for a second "rare event" from which a second new population can develop. The occurrence of this new focal change in a rare cell and its amplification by selection or differential growth seems to be no longer environment-dependent but appears to be self-generating. Because of this relative autonomy, at least with respect to added exogeneous agents, we consider this second rare event as the beginning of "neoplasia" and everything preceding this change as "preneoplasia." The subsequent steps are poorly understood and are discussed below (Figure 1).

Initiators

Until fairly recently, one of the most puzzling and confusing aspects of chemical carcinogenesis was the diversity in the chemical structure of carcinogens. This problem has now been largely resolved by the discovery of metabolic conversion of many carcinogens to highly reactive metabolites.[9] Although the principle was quite well established by the late 1960s, the many details concerning the specific forms of the metabolites are only now being clarified. The best known form of reactive moieties, generated from several different types of carcinogens, is the "electrophilic reactant," a positively charged molecule that reacts well with sites of electron densities in many different cellular components, including DNA, RNA, protein, glutathione, and probably also polysaccharides.[9] A minority of chemical carcinogens such as some nitrosamides (eg, alkyl nitrosoureas), *bis*-chlormethyl ether, and nitrogen mustard, are active *per se* and do not seem to require any metabolic conversion to more reactive metabolites.

Activation and Metabolism of Procarcinogens

The first major type of metabolic activation discovered was the conversion of an aromatic amine, 2-acetylaminofluorene (2-AAF), by *N-hydroxylation*

to an N-OH derivative.[9] This reactive product may in turn be esterified to a more reactive form, an N-O-ester, which is believed to be the ultimate type of carcinogen for some aromatic amines. The human bladder carcinogen 2-naphthylamine (β-naphthylamine) is a type of aromatic amine.

More recently, much effort has been placed on the clarification of the activation of benzo(a)pyrene (B(a)P) and other polycyclic aromatic hydrocarbons (PAHs). Many of these are easily generated by burning (pyrolysis), are therefore widely distributed in our environment, and are thought to be responsible, in part at least, for the carcinogenicity of coal tar products, oils, tars, etc. These compounds undergo *epoxidation* to form reactive epoxides, some forms of which are considerd to be ultimate carcinogens.[19] In the case of B(a)P and probably other PAHs, the initial site of epoxidation may undergo hydration to form a dihydrodiol, a reaction catalyzed by epoxide hydrolase. This inactive derivative in turn can be converted to another epoxide at a second site to form a dihydrodiol epoxide. These are considered to be the most likely ultimate carcinogens for at least some polycyclic aromatic hydrocarbons. This series of reactions for B(a)P is illustrated in Figure 2. Another potentially important carcinogen for humans that is subject to activation via epoxidation is aflatoxin B_1.[9] Aflatoxin undergoes oxidation at its 2,3-position; and this derivative, the 2,3-oxide, appears to be one form of ultimate carcinogen of this mycotoxin. Vinyl chloride is also activated via epoxidation.[9]

Very likely, these reactions with B(a)P, aflatoxin B_1, and vinyl chloride are illustrative of the probable activation of many other aliphatic, aromatic, or heterocyclic compounds, both carcinogenic and noncarcinogenic (eg, bromobenzene).

Other types of metabolic conversions also are considered to be important in carcinogenesis.[9] Nitrosamines are converted to highly reactive alkylating moieties, probably through oxidation. Aromatic or heterocyclic nitro compounds, such as nitrofurans and 4-nitroquinoline-N-oxide (4NQO) probably undergo initial *reduction* to hydroxyamino-derivatives. In the case of 4NQO, this form is considered to be an ultimate carcinogen.

Thus, several types of metabolic conversions to ultimate carcinogens are now known and offer interesting problems for detailed analysis of the chemistry, enzymology, modulation, and control.

For the majority of known conversions, the system most active in the cell is the "mixed function oxygenase" system (MFO), consisting of several cytochromes P450, NADPH cytochrome C reductase, and lipid.[20] This inducible system is located predominantly in the

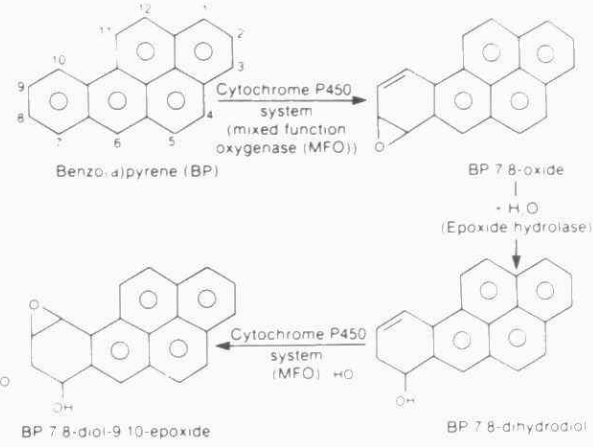

Figure 2—One pathway for the genesis of activated derivatives of benzo(a)pyrene via microsome, mixed function oxygenase system, and epoxide hydrolase.

microsomal fraction of the cell (endoplasmic reticulum, ER), although recent work increasingly points to a second comparable system in the nucleus.[21] In addition to these oxidative systems, reducing systems also exist for some precarcinogens, such as the aromatic or heterocyclic nitro compounds. Diaphorase or other NADPH or NADH reductases are widely distributed among different cells and are effective in reducing the nitro group to the amino or hydroxyamino derivative.

The liver is by far the most active and most versatile organ in the metabolism of precarcinogens and of xenobiotic agents generally. It and other organs show an ability to *detoxify* as well as activate potential carcinogens, and the ultimate fate of a chemical depends largely upon the balance between activation and inactivation, a balance that is easily modulated in major ways by drugs and other chemicals, age, nutrition, and hormones, as well as genetics.[22-27] For example, the carcinogenicity of several aromatic amines for the liver can be completely prevented by simultaneous exposure to phenobarbital or 3-methylcholanthrene, agents that induce many liver enzymes.[22,27,28] This phenomenon of resistance of the induced liver to some carcinogens may be of great importance to humans. Virtually all human beings in the Western world have levels of several xenobiotic agents, such as DDT, dieldrin, aldrin, PCBs (polychlorinated biphenyls), and dioxins in their adipose tissues. These agents as a group are very effective enzyme inducers in the liver. Also, many drugs induce various microsomal or other enzymes in liver or other tissues. Are these inducers making tissues more or less susceptible to the carcinogenic effect of other environmental agents?

It is considered probable that the organ or tissue distribution of the carcinogen-metabolizing enzymes

may play a role in the organotropism of carcinogens. Since there is an almost certain obligatory requirement of activation; and since the metabolic patterns affecting carcinogens and other xenobiotic agents are so variable from cell to cell or from organ to organ, the absolute activities of the various enzymes, and especially their relative balance, may play key roles in determining which organ, if any, will be a target for a particular carcinogen. An interesting example of modulation at this level is the liver–kidney axis with dimethylnitrosamine. This potent carcinogen normally induces liver cancer when taken in via the gastrointestinal tract. However, if an experimental animal is placed on a low-protein diet, the hepatic activation and metabolism falls off, making more carcinogen available for action on the kidney and changing the dominant cancer pattern from liver to kidney.[29]

A puzzling phenomenon relating to the metabolic activation of many carcinogens is its possible physiologic role. A reasonable interpretation of the major importance of "drug-metabolizing" enzymes is in the genesis of derivatives that can be excreted as such or that can be conjugated to facilitate excretion. These types of conversions occur with chemical carcinogens as well as with many other xenobiotics. What is the survival value for the host, if any, of metabolic activities that lead to highly reactive, more "toxic" derivatives, not less toxic? Are there naturally occurring substrates for these metabolic reactions which require "activation" for the performance of their metabolic roles?

Possible Molecular Targets for Ultimate Carcinogens

The molecular targets for the electrophilic reactants are many. To date, the bulk of the effort has been concentrated on DNA, with some interest in RNA and protein. In principle, many other molecular targets exist at the macromolecular and "micromolecular" levels. Because of the relative ease of relating changes in DNA to a permanent change in biologic behavior of cells, DNA has received the lion's share of attention. Since the first discovery of the chemical nature of an adduct of a carcinogen with nucleic acids in a target organ *in vivo*,[30] there has been an increasing interest in establishing the chemical nature and site of interaction with several different carcinogens.[16] As portrayed in Figure 3, virtually every potential site for interaction with DNA bases (as well as with phosphates) reacts with one or more carcinogens. In order to narrow down the possibilities for relevance to cancer, sites more likely to be involved in hydrogen bonding between bases (purines–pyrimidines) in DNA and thus to be involved in possible miscoding are often considered to be most relevant. Thus it is presupposed that the major or only biochemical lesion in DNA that is relevant to cancer initiation is a miscoding one. This judgment may not be justified, since the evidence is circumstantial and inconclusive. Conceivably, other types of alterations in DNA, beside miscoding, such as recombinations, gaps, translocation, and so on, may be important to the early events in cancer development.[16,31–34] In fact, Cairns has suggested that mutagenesis may be of only minor importance in the initial events in chemical carcinogenesis and that genetic transposition involving relatively large segments of the genome might be more relevant to the process.[34] The application of this newer technology to appropriate "clean" cell populations during the development of cancer is to be awaited with interest.

Another important aspect is the site of interaction of the ultimate carcinogen within the long DNA molecules. With the use of several different approaches, such as susceptibility to nucleases, separation of transcriptionally active from inactive DNA, or isolation of linker and nucleosome regions in chromatin, some of the interactions of carcinogens with DNA have been found not to be random. However, to date, no single region of high affinity has been found.[16] This interesting aspect of DNA as a probable target for carcinogens must await the further advances in DNA functional segmental fractionation, such as through the use of appropriate restriction enzymes and DNA cloning for specific genes in biologically well-defined preneoplastic and early neoplastic cell populations. Included will be the possible roles of histones and of the nonhistone chromosomal proteins in favoring certain regions over others.

DNA Repair

In view of the essentially irreversible nature of initiation with chemicals, and the apparent focal nature of the initiation process (see below), major emphasis at the molecular level is given DNA as a probable target in initiation. However, the evidence is largely circumstantial.

Great indirect support comes from the well-known high incidence of skin cancer in patients with xeroderma pigmentosum (XP). Most of these patients have some degree of interference with repair of DNA damaged by ultraviolet light. The latter is the major, if not sole, carcinogenic stimulus in these patients, and patients can be protected from skin cancer by careful avoidance of exposure to ultraviolet light.[35] Fibroblast cultures from patients with XP show increased sensitivity not only to ultraviolet light but

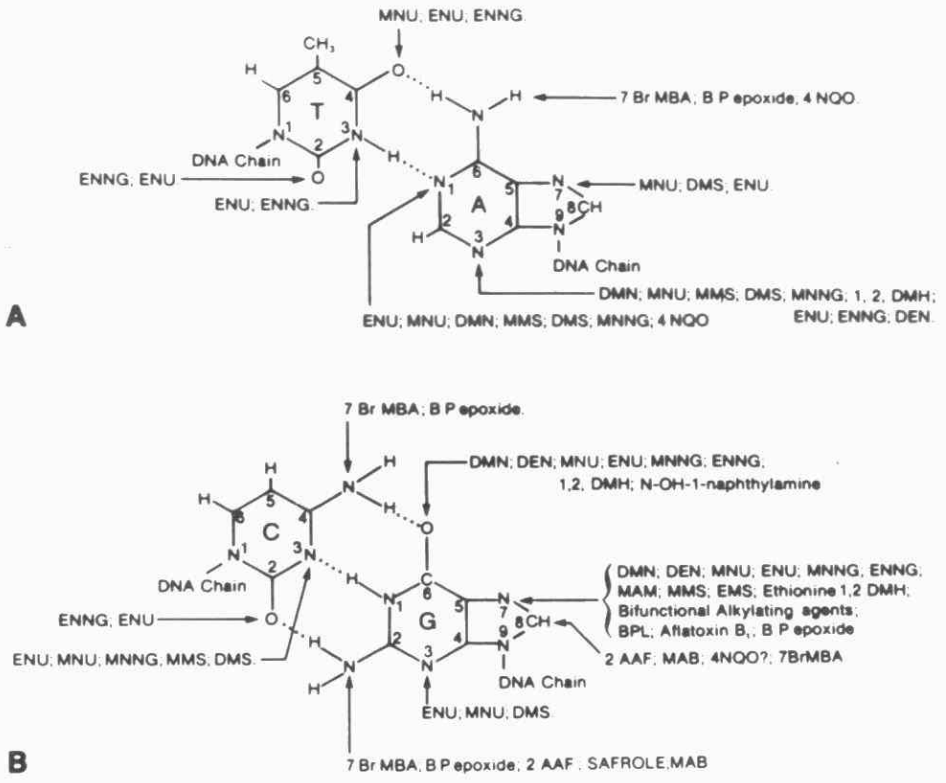
MNU; ENU; ENNG
7 Br MBA; B P epoxide; 4 NQO
DNA Chain
ENNG; ENU
ENU; ENNG
MNU; DMS; ENU
DNA Chain
DMN; MNU; MMS; DMS; MNNG; 1, 2, DMH; ENU; ENNG; DEN
ENU; MNU; DMN; MMS; DMS; MNNG; 4 NQO
A
7 Br MBA; B P epoxide
DMN; DEN; MNU; ENU; MNNG; ENNG; 1,2, DMH; N-OH-1-naphthylamine
DNA Chain
ENNG; ENU
DMN; DEN; MNU; ENU; MNNG; ENNG; MAM; MMS; EMS; Ethionine 1,2 DMH; Bifunctional Alkylating agents; BPL; Aflatoxin B1; B P epoxide
2 AAF; MAB; 4NQO?; 7BrMBA
ENU; MNU; MNNG; MMS; DMS
DNA Chain
ENU; MNU; DMS
7 Br MBA; B P epoxide; 2 AAF; SAFROLE; MAB
B

Figure 3—Known sites of adduct formation between activated forms of some carcinogens and the four common bases in DNA.

also to some chemical carcinogens and mutagens.[36] Other human diseases, eg, ataxia telangiectasia and Fanconi's anemia, also show repair deficiencies in DNA as well as increased risk for neoplasia.[37]

Thus, the bulk of evidence implicating DNA as a major target for initiation of the development of cancer comes from studies on human beings implicating ultraviolet light as the etiologic agent. This evidence is buttressed by a large amount of less definitive data on experimental chemical carcinogenesis and a general feeling that DNA repair may be a critical component in the development of cancer with chemicals. For example, there is a correlation under some conditions between the formation of O^6-methylguanine (O^6-MeG) in brain, persistence of this lesion, and the ultimate occurrence of gliomas.[38] This type of correlation is also present in liver and kidney under some conditions.[39] Another interesting example relates to the carcinogen 1,2-dimethylhydrazine (DMH). This compound is most active in inducing colon cancer and is not generally effective as an hepatocarcinogen. However, it does induce angiosarcoma of the liver. It has been found recently that the vascular lining cells of the liver are much less effective in the removal of O^6-methylguanine from their DNA than are the hepatocytes, an observation that correlates well with the "organotropism" of DMH.[40]

Yet other studies indicate that the genesis and persistence of O^6-MeG may be important but insufficient for cancer production with some nitrosamines or nitrosamides.[41] Also, in the liver, with some carcinogens such as 1,2-dimethylhydrazine and using a very early step in carcinogenesis as the end point, only DNA lesions removed within about 48 hours seem to be of major importance, and the persistent ones much less so, if at all.[42]

It is often theorized that our major protection against cancer resides in the efficiency with which we repair the DNA damaged by the many carcinogens and mutagens in our environment. As pointed out by German[43] and by Cairns,[34] since fibroblasts from patients with xeroderma pigmentosum show a defect in the repair of DNA damage by some chemicals[36] as well as by ultraviolet light, such patients should show an elevated risk for the development of cancer in organs or tissues other than the skin if chemical carcinogens are playing important roles in the genesis of many forms of cancer. Yet the available data show no apparent increased risk in patients with XP for cancer other than in the skin.[34] Patients with Bloom's syndrome do show such an elevated risk. Such observations are interpreted as evidence against a role for the many environmental chemical hazards in the etiology of human cancers generally, except in well-

established instances such as respiratory cancer in smokers and in occupational exposures.[34] However, an alternate hypothesis must be entertained, namely, that the role of repair may be quite different in the dynamics of induction of cancer with chemicals as compared with ultraviolet light.

The past 10 years have seen a large expansion of studies on DNA repair, largely in *in vitro* systems. These studies should lead to a much clearer delineation of the types of repair that *might* be occurring in carcinogenesis. What *does* happen remains poorly understood. Repair by base excision, by "long versus short patch" removal, by possible recombination or other forms of "postreplication" repair, by removing interstrand cross-links or other more complex forms of damage, or by other mechanisms are slowly being studied in many different systems *in vitro* and to a much lesser degree *in vivo*.[16,36] The enzymology, although difficult, is also slowly becoming clarified. In the case of methylating carcinogens, an interesting recent finding is the discovery of an enzyme in bacteria that removes the methyl group from the O^6-positive of guanine in DNA by transmethylation with its transfer to a S-containing moiety in protein.[44] This raises the question of whether some of the enzymes involved in repair of DNA damage perform physiologic functions that subserve normal cellular requirements other than repair.

An obvious failing in much of the attempt to correlate patterns of repair of DNA with carcinogenicity is the wide gap between the relatively advanced state of the chemistry, on the one hand, and the primitive state of the art in identifying and quantitating discrete biologic steps in the carcinogenic process, on the other hand.

Carcinogen Metabolism in Human Tissues

A gratifying aspect of recent studies on the early chemical and biochemical events in carcinogenesis is the overall similarity in patterns of activation and types of adducts seen in human tissues, as compared with the experimental animal tissues. Studies with some nitrosamines, benzo(a)pyrene, aflatoxin B_1, and other carcinogens (45–51) have shown that human tissues in general resemble one or more animal tissues in their patterns of activation and in the type of carcinogen-DNA adducts formed. Since the metabolism of any single carcinogen, including the quantitative aspects of adduct formation, varies in detail with the source and form of the activating system, such as whole-cell versus microsomes or microsomes–supernatant, it is not yet possible to attempt a highly detailed analysis of the biologic significance of the similarities and differences.

An interesting development that may help to clarify some aspects of this important phase of activation is the use of radioimmunoassays for specific adducts in DNA, especially with highly sensitive new approaches to radioimmunoassay.[52–54] Such detection of minute quantities of carcinogen-induced adducts in DNA opens up the possibility of studying the exposure patterns of individuals to carcinogens. This study, coupled with adequate medical examination and appropriate epidemiologic studies, might well offer new approaches to the ultimate problem of risk assessment. Although it is far too early to discuss such possibilities in other than general terms, it is important to emphasize that adduct formation is an index of *exposure*, not *initiation*, and that persistence of a chemical lesion in DNA is by no means synonymous with the development of cancer. As discussed below, initiation of the carcinogenic process is not an inevitable consequence of adduct formation. Nevertheless, the possibilities that seem imminent in this area of chemical carcinogenesis may allow the study of human carcinogenesis to enter a new phase of sensitivity with a new perspective.

Initiation

In the "classical" system of skin carcinogenesis in mice and rabbits, a single or brief exposure to a carcinogen induces some change in the tissues, called initiation, such that focal proliferations, called papillomas, can be made to appear by application of croton oil or other agents called promoting agents, which by themselves are noncarcinogenic or only weakly so. This phenomenon was more recently found also in several other organs such as liver, brain, mammary gland, colon, and urinary bladder.[5–7] Such focal proliferations (papillomas, polyps, hyperplastic nodules) in turn, are sites for further evolution to "neoplasia." Thus, initiation can only be defined so far in terms of a subsequent event in response to exposure to a promoting or selecting environment, ie, in biologic terms, not in chemical or biochemical terms. Initiation of skin, liver, mammary gland, and probably at most sites is largely permanent. Although some loss of intensity may occur with time, the general experience is that this biologic "event" probably persists for the lifetime of the animal.

These observations are most easily interpreted as reflecting some permanent change in the DNA in the rare cell affected during initiation *in vivo*. Whether the change is a mutation in a structural or regulatory

segment of DNA[55-57] or involves more complex rearrangements of larger segments of DNA, such as in transpositions,[34] remains unknown. Pertinent are the observations on transformation initiated with X-rays or with polycyclic aromatic hydrocarbons in which some change or changes other than a mutation in a rare cell appears to be involved as an early step in initiation *in vitro* with a highly selected cell type.[58,59] Until concrete evidence in support of one or another proposed mechanism becomes available, and until the molecular nature of the focal irreversible steps in normal development and differentiation is understood, it is preferable to denote the initiation and other rare focal changes during carcinogenesis as "rare events."[7]

One cannot equate activation of carcinogens leading to DNA-adduct formation with initiation. For example, many carcinogens such as B(a)P and 7,12-dimethybenzo(a)anthracene (DMBA) can be activated by liver to form adducts without initiating or inducing liver cancer in adult animals. However, if coupled with one round of cell proliferation, many can initiate[60-62] and can induce liver cancer.[63] *In vitro*, with different cell systems, cell proliferation early in the carcinogenic process has been shown to be required for transformation with X-rays,[64] some viruses,[65] and some chemicals.[66] Although suspected for *in vivo* carcinogenesis as well,[7,63] it has only been established for liver cancer formation[63] and very recently for liver cancer initiation.[60-62] Thus, as shown in Figure 4, initiation is at least a two-step process, a biochemical step that is repairable followed by a round of cell proliferation that "fixes" some change so as to make it essentially permanent. The cell proliferation can be generated by some primary mitogenic stimulus such as partial hepatectomy or by a chemical mitogen[60] or as a response to cell death.[67]

The mechanistic role of cell proliferation in initiation is not understood. The commonest hypothesis involves DNA synthesis or replication as somehow "fixing" damage in a daughter strand. While attractive, one must be aware of other possibilities related to the cell cycle such as variations in activation or inactivation, unusual availability of susceptible regions of DNA, or alterations in the repair processes at different times. With at least two models of liver carcinogenesis,[42,68] cell proliferation, to be effective, must occur within 1-3 days after the administration of the carcinogen.

The lack of a major degree of reversibility of the initiated state indicates that the very early carcinogen-induced altered cells are not recognized by the host in a manner that leads to their destruction. This raises serious doubts about the validity of the concept of immune surveillance at an *early* stage in chemical carcinogenesis,[69] as it has been proposed for the host control of the development of cancer.[70]

If the initiation process in the liver or elsewhere involves an occasional single or rare target cell, as is probable,[71-73] such isolated cells have yet to be characterized as to phenotype or genotype, since they have not been seen. Although many markers appear after the proliferation of the initiated cells, it is not known what role the cell proliferation plays in their appearance.

The probable requirement for cell proliferation for

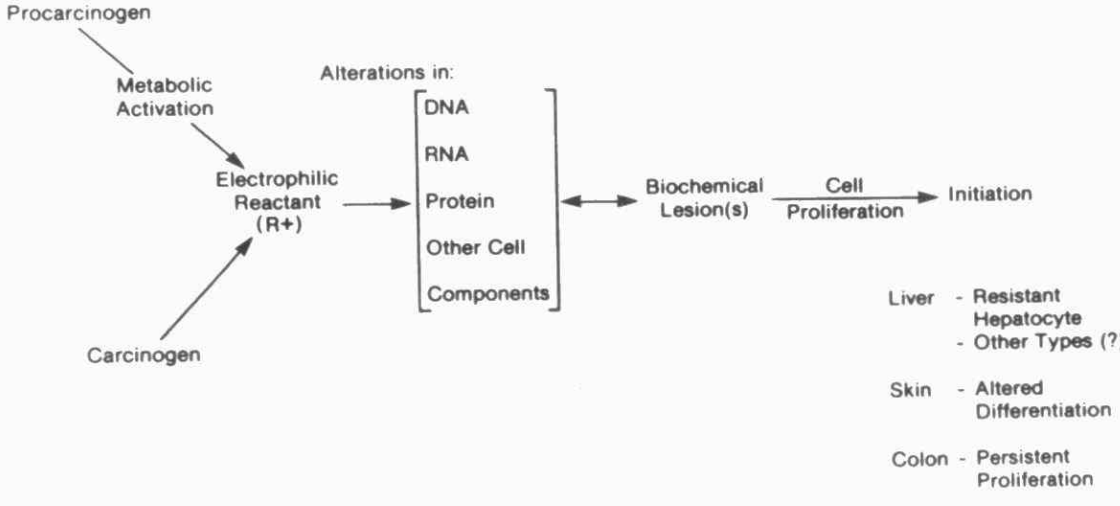

Figure 4—Diagrammatic representation of the first two steps in initiation of carcinogenesis—metabolism of the carcinogen with interaction with cellular constituents and cell proliferation.

intiation has important implications for the development of cancer in many sites in human beings. In the tissues showing continual proliferation (skin, bone marrow, gastrointestinal tract), initiation of carcinogenesis with chemicals could be dependent largely on the metabolic patterns of activation and repair, providing initiated cells are retained. However, in the nonproliferating tissues, such as pancreas, salivary gland, liver, kidney, urinary bladder, and brain, an important rate-limiting step for beginning the carcinogenic process may be cell proliferation. Thus, concomitant cell necrosis induced by viruses, toxic agents, parasites, dietary deficiencies, and so on, followed by cell regeneration, could be a major determinant for initiation of cancer in many sites. In addition, the presence of many proliferating cells is probably one basis for the susceptibility of the fetus and neonate to many chemical carcinogens[74] and could account for the peak in cancer incidence in the first decade. Some counterbalance for this could be the relatively poor development of the metabolic activating systems for some types of carcinogens in some species.[75,76] It is noteworthy and perhaps significant that the human fetus, unlike the rodent fetus, acquires the capability of activation of some carcinogens early in development[75] and thus may be at greater risk than some experimental animals for the development of cancer with chemicals.

Essential Nature of Initiated Cells. A most important question for initiation concerns the essential biologic nature of the initiated cells. What properties have they acquired that enable them, *as a group*, to be precursors for the ultimate development of cancer? What property or properties confer on these relatively few cells the *ability to be stimulated to form a focal area of proliferation*? The available evidence is against any conclusion that the initiated cells have acquired any autonomy of growth.[5,7] Without appropriate promotion by noncarcinogenic or carcinogenic agents, few if any postinitiation focal proliferations are seen in the systems so far studied.

In most systems, the properties critical to initiation remain unknown. In two continuously proliferating tissues, the skin[77] and the colon,[78] an early property of carcinogen-altered cells appears to be some disturbance in the programming or control such that the cells do not show the normal progression of differentiated properties. Whether these are "inherent" properties of the altered cells or a reflection of some change in the local environment has not been established.

In the liver, three properties of initiated cells have been observed. The first, the appearance of enzyme deficiencies or excess or other biochemical changes, have been used to identify putative preneoplastic hepatocytes[62,79,80] However, so far no hypothesis has appeared implicating any one or more of such changes in mechanisms of carcinogenesis. The second property, resistance to the inhibitory effects of carcinogens on cell proliferation, has been shown to be that of *one type of initiated cell* in which a mechanism for focal proliferation can be formulated (*differential inhibition*).[81] An assay for the quantitative assessment of such resistant hepatocytes has been used to study a variety of properties of initiation and initiated cells.[81] A third functional property has been proposed—retention of a response to mitogens by initiated cells and loss of such response by the majority of surrounding cells on repeated stimulation.[82] This approach also can be used as a model for a mechanism of promotion (*differential stimulation*).[83]

In this context, it is attractive to consider that many different types of initiated cells may be induced by exposure to an active carcinogen (Figure 5) and that the nature of the cells selected or "promoted" may be a property of the promoting or selecting environment. Conceivably, each target organ or tissue for a carcinogen might well have a spectrum of types of potentially initiated cells, but the cells selected or promoted may reflect the mechanism of promotion appropriate for that particular tissue or organ (see next section). Since a round of cell proliferation seems to be an essential step for initiation, the only

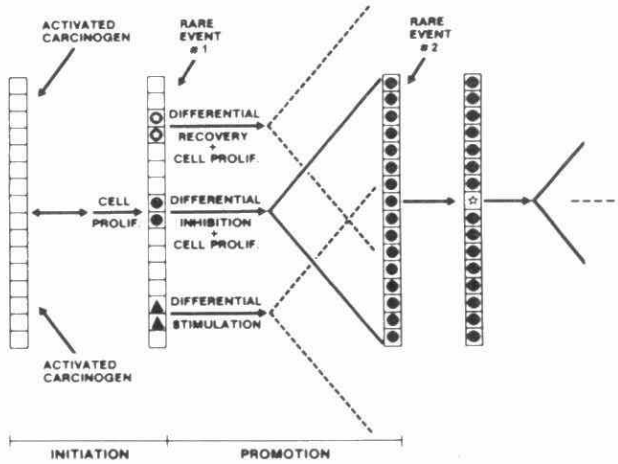

Figure 5—Diagrammatic representation of initiation and promotion, as viewed with the rare event-selection hypothesis. The different symbols represent different types of functionally altered hepatocytes. Although only three general types of initiated cells are included (○ ● ▲), it is possible that many more types may be induced by an initiating dose of a carcinogen. Conceivably, only some types can be promoted or selected by environments appropriate to each particular tissue or organ. According to this hypothesis, amplification (or expansion) of appropriately altered hepatocytes is the first prerequisite of promotion in the liver.

cells that are targets for the initiating effects of a carcinogen would be those that are cycling or those that enter the cycle before repair of the critical molecular lesions is complete.

Thus, initiation of chemical and some other forms of carcinogens can be viewed as the *induction* of altered cells that can be *selected* by an environment appropriate for those cells and for the tissue or organ in which they are induced.

Whether some or all initiated cells have, in addition to a "handle" for selection, properties that relate directly or intimately to cancer remains to date unknown. At one extreme is the hypothesis that the initiation process does nothing relevant to cancer other than allow a rare cell or group of cells to develop into a focal proliferative lesion and that all subsequent steps related to cancer are acquired at later times. At the other extreme is the hypothesis that the initiating dose of a carcinogen imparts some more direct cancer-related property or properties to the initiated cells, and it is such properties that play a role in determining which of the many preneoplastic lesions progress to cancer. The obvious challenge to cancer research is to devise experimental approaches to test these hypotheses.

Prehn[84] and others have periodically suggested that a carcinogen or carcinogenic stimulus might not *induce* an altered cell during initiation but rather might encourage the selection of a preformed cell already present in the target tissue. Since promoting agents or environments for the skin or liver often do not lead to the appearance of preneoplastic or neoplastic lesions in the absence of an initiating exposure to a carcinogen, it does not appear likely that initiated or altered cells exist preformed in most models of carcinogenesis. In the many studies on skin and in the increasing number on liver, exposure to a carcinogen under "initiating conditions" is a prerequisite for carcinogenesis except in a rare strain of susceptible animals.[71,85-90] However, the unusual susceptibility of a rare preformed cell to the inducing or initiating effects of carcinogens cannot be ruled in or out with the currently available methods of cell analysis in tissues. A negative conclusion concerning the selection of a preformed altered cell by a carcinogen *in vitro* has been reached by Heidelberger.[91]

Thus, in several different systems, it appears that exposure to a carcinogen under initiating conditions *induces* rather than *selects* altered target cells, although an unusual or rare susceptibility of some preformed cells to such an inducing effect has not been ruled out. If, as seems probable, many different types of initiated cells may be induced by exposure to an activated carcinogen, the existence of a small population of preformed cells that are peculiarly programmed for susceptibility becomes much less likely.

In the liver, the initiated resistant cell that can be selected in the Solt-Farber model appears to be randomly distributed without any apparent predilection for one or other zones of the liver acinus.[71] This plus other considerations[7] make it highly unlikely that the target cell in the liver for carcinogens is a hypothetical stem cell. Whether such hypothetical stem cells exist and are targets in many other tissues remains unknown. In those tissues in which a reserve of proliferating cells are considered to be stem cells (eg, bone marrow, skin[?]), it is unknown whether such cells are peculiarly susceptible to the initiating effects of a carcinogen. Naturally, in such tissues, if a round of cell proliferation is obligatory for initiation, as is very probable, the replicating cells would be the exclusive targets in carcinogenesis. However, in the skin at least, situations exist in which cell division appears in suprabasal layers of the epidermis. These cells recruited for proliferation also might well be susceptible to initiation of carcinogenesis.

Although the majority of chemical carcinogens fall well within the current paradigm of initiating effects being related to some form of DNA damage, there are known carcinogens that appear to be exceptions. A growing list of hypolipidemic agents,[92] several pesticides, herbicides and other xenobiotics,[93] and at least one drug, methapyrilene[94] have so far not been shown to generate mutagenic or other DNA-damaging effects. Is this merely a reflection of deficiencies in our technology, or are there pathways to cancer that do not involve DNA damage of exogenous origin as essential early steps in the process?

Promoters and Promotion

The term "promotion" is often used for the process whereby "neoplastic development," "tumor formation," or "cancer development" is accelerated or encouraged in a tissue that has been exposed to an initiating dose or doses of a carcinogen.

Promoters

Early in the recent history of chemical carcinogenesis, studies by Rous, Berenblum, Mottram and their respective coworkers led to the discovery of a model for chemical carcinogenesis that has laid the major foundation for our current views. The observation that a noncarcinogen, croton oil, could stimulate tumor formation in the skin following a brief initial exposure to a carcinogen naturally encouraged a chemical "attack" on croton oil. Hecker[95] and Van Duuren[96] and their colleagues discovered that esters of the

diterpene phorbol, isolated from croton oil, have very potent promoting effects on mouse skin. The past several years has seen the appearance of a large number of reports on many different effects of these and similar agents from related plants such as ingenol and resiniferol.[97] Many biochemical, metabolic and biologic effects are induced in many different normal cells by the phorbol esters and in the vast majority of cases, the intensity of the particular effect parallels closely the efficacy of the compounds as promoters of skin papillomas in mice following an exposure to a single dose of DMBA or a similar carcinogen.[97,98] These effects include changes in morphology, microtubule polymerization, cell proliferation, enzyme induction, polyamine synthesis, phospholipid synthesis, membrane structure and function, adenosine triphosphatase (ATPase), release of prostaglandins, inhibition and sometimes stimulation of differentiation, and many others in a wide variety of normal cells and cell lines. Many of the effects are considered to be pleiotropic.

Very recently, using a less active phorbol ester, ^{3}H-phorbol dibutyrate, Blumberg and Shoyab et al[97,99] have found that membranes of fibroblast cultures and mouse epidermal cells contain a high affinity receptor for many phorbol esters, including one of the most active, 12-O-tetra-decanoyl-phorbol-13-acetate (TPA). As yet the "natural" metabolites for the receptors have not been identified. Given the wide diversity and the large number of effects seen in treated cells, it may be that more than one type of receptor is involved.

The current studies on phorbol esters and related compounds offer some exciting new tools for the study of a variety of aspects of cell structure and function. At a minimum, they should lead to a better understanding of how the binding of a chemical to receptors triggers a whole array of biologic responses and what known and unknown pathways are used to effect the changes in cell structure and function. In addition, since some of the vitamin A analogs, retinoids, antagonize selected effects of the phorbol esters and also delay or inhibit cancer development in several systems,[100] where do the molecular pathways for the effect of these two classes of agents intersect and interact? These are most interesting areas for studies of fundamental cell biology.

However, as yet it is impossible to relate the findings *in vitro* to chemical carcinogenesis *in vivo*. Given the array of phenomena induced by the phorbol esters, how does one select relevance to promotion?

A major problem concerns the matching of the target cells *in vivo* and *in vitro*. In the intact animal, an active promoter does not induce focal proliferations such as papillomas in the normal skin, but only after initiation. However, many skin promoters do induce general hyperplasia of the epidermis in uninitiated animals. Studies in several laboratories have shown that a general stimulation of cell proliferation is insufficient for the selective or differential stimulation of initiated skin to form papillomas.[86]

The Process of Promotion or Selection

Definition. Operationally, the term "promotion" is often used for the process whereby "neoplastic development," "tumor formation," or "cancer development" is accelerated or encouraged in a tissue that has been exposed for a relatively brief period to an initiating dose of a carcinogen. As with the term "initiation," this type of definition is not very useful for any stepwise analysis of carcinogenesis, since it does not include limits to end points and incorporates in a vague manner both early and late steps in the multistep process of cancer development.

A more useful definition is as follows: *promotion is the process whereby an initiated tissue or organ develops focal proliferations, one or more of which may act as precursors for subsequent steps in the carcinogenic process.* The definition appears to be valid at least in the skin and the liver, two sites in which the sequential analysis of cancer development is the most advanced. This definition limits the term "promotion" to a single biologic phenomenon and to one that may be quantitated by the use of at least three parameters: number, size, and rate of appearance of focal proliferations. This definition also implies that an essential element of promotion is the *selective stimulation* of only a very small proportion of the cell population in the original target tissue. In other words, a promoting environment supplies the *motive force for the proliferation of initiated cells in a selective fashion.*

Properties. In the skin and in one model in the liver, "*the resistant cell model*,"[71,81] the application of a strong promoter or promoting environment produces, as a very early phenomenon, focal proliferations, the number of which is proportional to the dose of the initiating carcinogen used. In the liver model, a single, relatively brief period (2–3 weeks) of focal proliferation, resulting in the appearance of liver cell nodules (hyperplastic nodules), is sufficient to set in motion the remainder of the process leading to liver cell cancer in about 70–80% of animals.[71,101,102] In one or very few nodules new focal

cellular proliferations appear that generate "nodules within nodules," and this process seems to repeat itself until obvious malignant hepatocellular carcinoma appears. Thus, the termination of the promoting operation occurs long before the second rare event appears; ie, an external promoting environment is only necessary for a relatively brief period during the preneoplastic phase of carcinogenesis.[7]

In the skin, even with very potent promoters such as some of the phorbol esters, repeated application is necessary before focal proliferations (papillomas) become easily recognized. However, as has been repeatedly reported,[7] the application of the promoter can terminate *before* the appearance of the next obvious tissue change, the focal alterations in one or a few papillomas ("rare event" number 2).[5,7,103,104]

In other models of liver carcinogenesis[87,88,105,106] and of carcinogenesis in other organs[6,7] the promoting environment has been imposed for many months, and the minimal time for adequate promotion has yet to be reported.

The demonstration of a "material continuity"[5,7] or a physical or cellular link between initiated cells and presumed subsequent steps in the stepwise evolution to cancer is an absolute prerequisite for any model to be useful in the study of the sequential events in carcinogenesis, and this link is lacking in most models.

In the skin, the focal proliferations appearing during promotion have classically been called tumors or papillomas. Similarly in the liver, the focal proliferations or hyperplastic nodules have been designated by some authors as adenomas[107] or more recently neoplastic nodules.[108] Since most of the so-called papillomas and adenomas or neoplastic nodules undergo regression or maturation (remodeling) on termination of the promoting environment, I consider their designation as benign neoplasms inappropriate. As long as persistence or continued proliferation is environment-dependent and reversible (Figure 1), I consider the focal proliferations to be largely hyperplastic in nature and not neoplastic. Thus, it appears reasonable to designate the lesions that appear prior to rare event number 2 as *preneoplastic* and essentially *hyperplastic*.

As observed in experimental *in vivo* models in common use today, cell proliferation is an essential phenomenon in promotion. It is therefore to be anticipated that many biochemical changes seen in the different phases of the cell cycle,[109] such as an increase in ornithine decarboxylase activity, will be seen. Whether one or more of such changes in enzymes, cell membrane, DNA organization, and so on, will play a *special* role in promotion over and above that related to the cell cycle *per se* remains an interesting challenge.

Essential Nature and Possible Mechanisms. According to this formulation, promotion consists, as a minimum, of the expansion or amplification of carcinogen-induced initiated cells to form focal proliferations, one or more of which develop further changes that may lead to cancer. Thus, an essential feature of promotion is the creation of a mitogenic environment that imposes a differential effect on initiated cells vis-à-vis the surrounding cells.

If the first carcinogen-induced initiated cells have not acquired any autonomy of growth or proliferation, as appears to be the case,[7] how can such cells be encouraged to produce focal proliferations? At least four possible overall ways come to mind: 1) *differential inhibition*; 2) *differential stimulation*; 3) *differential necrosis with regeneration*; and 4) *differential recovery*.

DIFFERENTIAL INHIBITION: If a general stimulus for cell proliferation (eg, a promoter) is applied, and cell proliferation is inhibited in the surrounding cells but not in the initiated cells, a focal proliferation of the initiated cells would be expected. Such an overall biologic mechanism could well lead to nodules or papillary growths. If the stimulus is reasonably intensive and if the inhibition is marked, rapid proliferation of initiated cells would be anticipated. This appears to be the general mechanism for the rapid induction of hyperplastic nodules in the "resistant cell model" of liver carcinogenesis.[71,90]

Since most carcinogens inhibit cell proliferation and/or DNA synthesis to a greater degree in many uninitiated cells than in preneoplastic or neoplastic cells (see Farber[110] for references), differential inhibition is a likely phenomenon in at least some tissues or organs that are exposed to carcinogens continuously or even intermittently for a relatively long period. Chronic exposure to carcinogens is commonly seen in most instances of cancer in nature.

DIFFERENTIAL STIMULATION: Differential stimulation has been proposed as the overall mechanism for liver cancer induction by at least two xenobiotic enzyme inducers, α-hexachlorocyclohexane (α-HCH) and cyproterone acetate (CPA).[82] These compounds are mitogenic for hepatocytes. However, the uninitiated hepatocytes lose their response after one or a few cell cycles, while the initiated cells continue to respond, thus generating nodules.

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A more useful definition is as follows: *promotion is the process whereby an initiated tissue or organ develops focal proliferations, one or more of which may act as precursors for subsequent steps in the carcinogenic process.* The definition appears to be valid at least in the skin and the liver, two sites in which the sequential analysis of cancer development is the most advanced. This definition limits the term "promotion" to a single biologic phenomenon and to one that may be quantitated by the use of at least three parameters: number, size, and rate of appearance of focal proliferations. This definition also implies that an essential element of promotion is the *selective stimulation* of only a very small proportion of the cell population in the original target tissue. In other words, a promoting environment supplies the *motive force for the proliferation of initiated cells in a selective fashion.*

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DIFFERENTIAL NECROSIS WITH REGENERATION: Differential necrosis with regeneration is proposed for some promoting environments in liver and elsewhere. Since focal hyperplastic lesions (nodules) are resistant to

the cytotoxicity of carcinogens and other toxic agents,[111] the selective induction of necrosis in the tissue surrounding the foci of initiated cells followed by regeneration would maintain the surrounding tissue in equilibrium, while the growth stimulus would produce progressive growth of the resistant nodules. The promoting effect of a choline-deficient diet[105,106] on liver carcinogenesis may well be of this nature.

DIFFERENTIAL RECOVERY: Differential recovery is a proposed biologic mechanism for some systems in which both the initiated cells and surrounding cells respond equally, but the initiated cells fail to return to the resting condition as quickly or as completely as do the surrounding. This delay or block in recovery is seen in liver hyperplastic foci and nodules,[81] and might well be a property of other initiated cells also. Conceivably, the active phorbol esters or other effective skin promoters might operate through such an overall mechanism.

Since each one of these four suggested mechanisms would probably operate through different biochemical or molecular mechanisms, it would appear to be important to establish the overall phenomenon before focusing on one or more molecular hypotheses.

Another critical question concerning promotion is whether focal or differential cell proliferation is sufficient to set in motion the chain of events leading to cancer or whether additional effects of carcinogens or of promoting environments, such as gene activation, or other biochemical cellular modulations in the amplified or expanded initiated cells are required. In other words, is *amplification of an initiated cell population sufficient* or is *amplification plus cellular modulation* essential?

To my knowledge, this crucial question has not been posed experimentally, since the assumption is generally made that modulation (gene activation?) is needed for progression and evolution to cancer. This prejudgment appears to be premature. This is particularly evident when one realizes that the amplified initiated cell population does not progress to cancer *en masse* but rather appears to provide a population of appropriate size and at sufficiently high risk for the occurrence of the next rare event (number 2).[7]

Fate and Options for Focal Proliferations. It is well known that the majority of papillomas in the skin induced by a phorbol ester after application of a single dose of a carcinogen undergo regression with only a minority persisting.[85,86,104] A similar phenomenon has been seen in the liver and urinary bladder[7] and possibly in the colon.[112]

In the liver and in the skin, at least two populations of focal proliferations (nodules and papillomas) have been identified—a majority that regress or mature (remodel) and a minority that persist. Subsequent focal atypical and neoplastic cellular changes have been found in an occasional persistent nodule or papilloma.

In the liver, new insights are appearing from studies on the resistant cell model.[102,113-115] Since a sequence of early preneoplastic hepatocyte lesions now can be followed (Figure 6), it is becoming apparent that there are at least *three distinguishable focal lesions* that appear sequentially. The very smallest foci, composed of only a few cells (perhaps up to 8 or 16 cells), are generally low in epoxide hydrolase activity but high in γ-glutamyl transpeptidase.[102] These small lesions persist for long segments of the animal's life span, may show a slow increase in size and do not seem to undergo any remarkable change in appearance. This is also the experience of Scherer and Emmelot,[116] Sirica et al,[117] and Peraino et al.[87] When livers containing these very early foci are selected (or promoted), the foci rapidly grow into nodules within a matter of days.[71] While still microscopic, the enlarging foci now become much more active for epoxide hydrolase.[102] When these reach a size of about 1 mm or so within 2–3 weeks, they retain their high activities of epoxide hydrolase and γ-glutamyl transpeptidase and now show one of two overall fates.[102] The majority (90–95%) undergo remodeling toward normal-appearing liver, with the gain of negative markers and the loss of the positive ones.[102,113,115] The remodeling nodules can be identified very early. However, the extent of remodeling is quite variable; some nodules show a rapid change (1 month or so), others a slow change (a few months), and the majority fall in between. A small minority, about less than 5%, show no remodeling but persist as unremodeled nodules. Basophilic nodules and ultimately metastasizing hepatocellular carcinoma can be seen arising inside a few such nodules. The persistent nodules show labeling indices of 2–3%, as compared with the indices in the remodeling nodules and surrounding liver, of 0.1–0.2%.[115]

Thus, it appears that more refined steps may be identified early in carcinogenesis with the use of a much better synchronized system (Figure 6). Because of the intensity of the selection pressure (the promoting environment), all the responding resistant hepatocytes behave pretty much in unison, producing a moving cohort up to the time of the appearance of the visible hyperplastic nodule. Within 3–4 weeks, the nodules become sufficiently large to be mechanically "shelled out" from the surrounding liver.

For biochemical, metabolic, and dynamic biologic

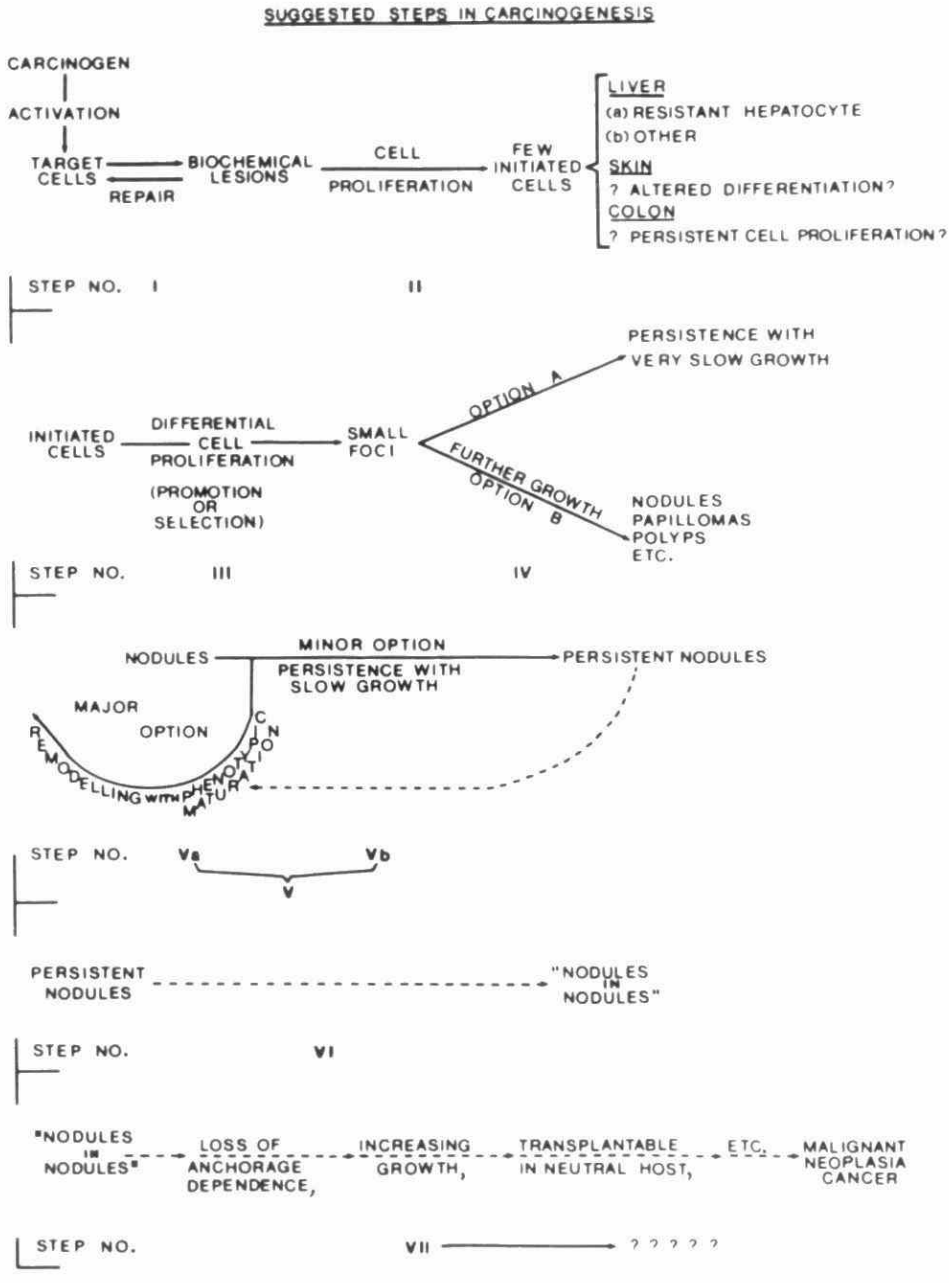

Figure 6—Current view of the sequence of steps during the genesis of hepatocellular carcinoma with chemicals. This concept was derived from the study of the available models of liver carcinogenesis, with emphasis on the resistant hepatocyte model.

studies, grossly visible lesions that are synchronized are essential. Most of the models of liver carcinogenesis do not generate such lesions and therefore are not particularly useful for these types of studies. This is regrettable, since the limitations that exist in a model that requires microscopic examination for analysis are serious.

A critical issue that has not yet been analyzed is the basis for the difference between the majority of focal populations that undergo some form of regression or remodeling and the minority that persist. In turn, are all the persisting nodules capable of acting as sites for further steps to cancer, or is this population also heterogeneous in this respect? With regard to regression versus persistence, is this option built in at the time of initiation, or is it a reflection of the intensity of a continuing environment that favors persistence by stimulating cell proliferation? If the latter is valid, the degree of persistence is the result of a stochastic phenomenon and not predetermined by exposure to the carcinogen.

With regard to the question of equivalence or other-

wise of all persisting lesions, conceivably they may *all* be capable of acting as precursors for cancer, but the rates are different and the animal succumbs to one cancer before others appear.

Another desirable property of models of carcinogenesis is the clear-cut evidence that each proposed lesion is truly a step in a proposed sequence. This requires that a physical link be established between the different populations. This is readily shown in the papilloma in the mouse skin, in which further progression to cancer can be seen. In the liver, only the resistant cell model has shown this sequence: resistant initiated cell → focus → nodule → metastasizing hepatocellular carcinoma[71] (Figure 6). Unfortunately, no model is available that allows an unequivocal study of the cellular metabolic and structural changes integral to preneoplastic, neoplastic, and malignant neoplastic transformations. In every available model, the number of putative preneoplastic focal proliferations is always larger, sometimes by a factor of 10 to 100, than the number of focal proliferations undergoing further steps in the process. Improved models are clearly needed if the preneoplasia–malignant neoplasia series of steps is to be studied scientifically.

Naturally Occurring Promotion or Selection. There is a strong suspicion on the part of some investigators that many tissues or organs create a "physiologic" promoting or selecting environment. The most provocative evidence in this respect relates to carcinogenesis in the colon or the urinary tract. In each of these locations, diversion of the normal stream of movement of contents has been found to lead to a decreased incidence of neoplasia with known carcinogens.[118-121] Also, there exist in the literature many examples in which cancer can be induced by a single exposure to a large dose of carcinogen, a situation that might well be dependent upon an interplay between a "physiologic" promotion and the appropriate metabolic and structural changes in a target organ or tissue. For the liver, the continual exposure to many compounds from the intestine, and for the skin, exposure to ultraviolet light, could be playing selecting roles in carcinogenesis.

There is a growing feeling that one of the major factors in determining cancer development is the presence and intensity of promoting environments.[122] In addition to some specific agents, such as phenobarbital, butylated hydroxytoluene, and polychlorinated biphenyls for liver,[79] prolactin for the mammary gland,[123] and bile acids for the colon,[124,125] diet has been found to influence carcinogenesis in several systems.[1,2] However, the specific roles of individual dietary components and of their balances remain to be established in most instances.

It is noteworthy that the imposition or application of a promoting environment need not be continued until obvious neoplasia develops.[5,7] Rather, as already mentioned, the promoting effect seems to be necessary only to induce a persistent focal proliferative lesion, such as a nodule, a papilloma, or a polyp; and the further evolution to neoplasia and cancer appears to be "self-generating."[5,7,81,85]

There are several examples in human beings where synergism or promotion may be operating. For example, it is well documented that exposure to asbestos, nickel, or uranium may only be associated with a relatively low risk of the development of lung cancer. However, when coupled with cigarette smoking, the risk becomes extremely large, much larger than with smoking alone.

These may well be striking examples of a common phenomenon in the development of cancer. There are at least two known sites for synergistic effects. At initiation, one agent might allow carcinogenesis to begin by providing an episode of cell injury followed by cell proliferation. Such an effect might be anticipated for those organs or tissues that show no regular cell proliferation. A second site is likely to be promotion. It is becoming evident that the initiating and promoting effects of carcinogens may have quite different dose-response characteristics and different kinetics.[61] The combination of a "good initiator, poor promoter or selector" and "poor initiator, good promoter or selector" may be highly efficient as a carcinogenic regimen when contrasted with either carcinogen alone.[126] Given the ubiquity of xenobiotics, it would seem particularly timely for more intensive studies on the combined effects of xenobiotics in carcinogenesis.[126]

Subsequent Sequences – Progression

As already indicated, there is considerable evidence that one or more of the focal proliferations appearing during promotion or selection are the sites of further change in one or a few cells. These few cells seem to have acquired some autonomy, since they apparently proliferate without the evident need for an external influence. It is believed that this process of appearance of rare change (rare event) and selection repeats itself until the organism dies with advanced malignant neoplasia (Figure 1).

In man, one consistently sees in many organs several types of putative precancerous lesions, such as atypical hyperplasia, dysplasia, and carcinoma *in situ*.[127,128] These lesions appear to occur at later times in carcinogenesis than do the focal proliferations discussed above. How these sets of lesions relate to each other and to altered biologic behavior of cells during

in vitro transformation (eg, atypical growth, growth in soft agar, acquisition of infinite capacity for growth) is not clear and remains potentially a very fruitful area for study.

Another important aspect that is now beginning to receive attention is the reversibility of steps in chemical carcinogenesis. There is considerable evidence experimentally and also in humans that some focal proliferative lesions, such as hyperplastic nodules in the liver (and their probable human equivalent, liver "adenomas" seen rarely in women on oral contraceptives), polyps in the colon, and papillomas in skin can undergo regression or remodeling with normal differentiation.[129] This area is becoming of particular interest in view of the reported efficacy of vitamin A analogs, such as *cis*-retinoic acid, in preventing or delaying the development of cancer in several systems initiated with chemical carcinogens,[100] such as the bronchus and the urinary bladder.

The development of models for the sequential analysis of the early changes in the carcinogenic process is most encouraging. However, since these changes are dependent on the environment, and the later ones seem not to be, it may be more difficult to develop synchronized models for the later steps in the process.

In the liver and in the skin in experimental animals and in many sites in humans, it has been shown that presumptive precancerous lesions or even cancerous lesions can arise in an area of focal proliferation characteristic of the early sequence in the neoplastic process. This has been reported many times in the skin. In the liver, frankly malignant lesions or histologically atypical nodules have been found to arise in hyperplastic nodules induced by ethionine, aramite, diethylnitrosamine, or 3′-methyl-4′-dimethylaminoazobenzene.[71,130-133] Thus, clearly the suggested role of some of the early focal lesions as possible precursors of cancer is well grounded. Any suggested sequence that bypasses or omits the hyperplastic nodule, the papilloma, or similar analogous lesions as *one site of origin of cancer* ignores this unequivocal evidence.[134]

Of course, great caution must be used in assigning an *exclusive role* as cancer precursor to the preneoplastic focal proliferation. Over the years, investigators have brought forth evidence that cancer can arise in at least some organs (skin, colon, liver, etc.) without the occurrence of focal proliferations as precursor lesions. It is certainly very possible that cancer can develop through different sequences.[7] However, if such proposed sequences are to be studied and analyzed in detail, appropriate *analyzable* models must be developed. To date, very few, if any, models that can be subjected to critical analysis via hypothesis testing have been reported. An obvious problem in model design that makes a negative finding uninterpretable is the fact that new focal proliferations always grow more quickly than their precursors. Naturally, under these conditions, any proliferating cell population very often destroys rapidly its precursor population. Thus, the absence of a visible precursor at any moment in time does *not* establish that such a precursor did not exist. Clearly, a large expanded precursor population, such as a large nodule, is necessary in most instances in order for one to observe a new population arising in an older one. This serious experimental complication has been overlooked in many studies that claim the absence of a precursor for any cell population acting as a step in a sequence leading to cancer.

Transfection

An interesting recent development is the induction of transformation by the transfer of DNA or chromatin to susceptible cell lines. DNA from several types of cells transformed *in vitro* or *in vivo* with different carcinogens was shown to induce transformation.[135,136] Of great interest is the observation that DNA from normal cells was equally effective. These new approaches to chemical carcinogenesis open up many areas of exploration, including the question of whether the information contributions of the DNA are positive, ie, code for an identifiable component important in cell transformation or negative, ie, disorganize the host genome in an appropriate manner. Clearly, the possibilities opened up by these interesting studies on transfection are provocative and intriguing.

Diet and Carcinogenesis

There is increasing evidence that the dietary composition may influence cancer incidence in some sites such as colon, breast, and endometrium.[137-139] The mechanism(s) through which diets exert their influence are not well understood. In some instances, carcinogens occur in the food as natural substances, as contaminants (eg, aflatoxins), or as products of food preparation (eg, pyrolysis). Also, dietary alterations are known to modulate carcinogen activation.

In other instances, micronutrients have been shown to play a role in the endogenous formation of carcinogens. The past 10 years have seen a revival of the concept of endogenous origin for some carcino-

gens. This idea was proposed in the 1930s in relation to cholesterol or other sterols but was progressively abandoned as the evidence failed to materialize. The resurrection of this idea assumes a new form in the genesis of nitroso-compounds from nitrite and secondary or tertiary amines.[140] Dietary amines or drugs such as oxytetracycline or chlorpromazine can react with nitrous acid generated in the stomach from nitrite to form nitroso-compounds. Some of these are carcinogenic in experimental animals. The formation of such nitroso-compounds can be effectively inhibited by dietary ascorbic acid[141] and by vitamin E.

This general model is being explored in human colon cancer.[142] The hypothesis states that carcinogens can be generated in the feces and that the levels of these, as measured by mutagenic actions, is influenced by the dietary composition, including vitamins C and E and fiber content.

Chemotherapy and Carcinogenesis

Many of the most effective chemotherapeutic agents for cancer are carcinogenic. For treatment of cancer in the age range above 50–60 years, the significance of the carcinogenic risk is clearly minimal, given the long "latent period" for cancer development. However, in children and young adults, the development of second cancers years after the effective treatment of the primary cancer is now becoming a recognizable problem.[143–145] Over 25 drugs used in chemotherapy have been found to be carcinogenic in animals and some also in humans.

The magnitude of the risk for the many treatment regimens is not fully known. However, for some, the risk of second primary cancers varies from 7 to 12%.[145] These figures include some risk factors, possibly genetic, that favor second or multiple primary cancers quite aside from the therapy. However, there is an additional risk from the chemotherapy itself. It is naturally anticipated that this risk will increase as current types of chemotherapy for cancer become more successful. It is not too much to hope that, as mechanisms about how chemicals induce cancer and how chemicals kill cancer cells are clarified, ways will be found to prevent the carcinogenic effects without interfering significantly with their efficacy in treating cancer.

It is perhaps noteworthy in this context that a common problem in cancer chemotherapy is the appearance of resistant cancer cells. Since resistant cells are also produced by carcinogens during carcinogenesis and since such resistant cells constitute one form of initiation, it is conceivable that the induction of resistance to therapy and of cancer might be related.

Model Systems for Chemical Carcinogenesis

A development of major import in chemical carcinogenesis is the use of new model systems, many exclusively *in vitro* and some combined with *in vivo* approaches.

In Vitro *Cell Cultures*

Fetal and adult cell cultures have been shown to be transformed by a wide array of chemical carcinogens.[91] As with the whole animals, appropriate metabolic conversion to active derivatives is required for procarcinogens to become effective, and various ways to accomplish this have been devised. Human cells resisted such transformation for several years. However, the past 3 years have seen positive results in some human cell systems.[146–150] The apparent resistance was in part technical and in part related to the end point measured.

Overall, there are basically two types of systems being used.[91] One type utilizes primary cultures of Syrian hamster or other embryo cells, often plated on a feeder layer of lethally irradiated cells. The second type utilizes a variety of permanent cell lines such as C3H/10T½ cells. These cell lines are often aneuploid and cannot be considered completely "normal" cells, since they grow permanently. Despite this, such cell systems are very useful for the study of many aspects of carcinogens and carcinogenesis.

The first unequivocal demonstration of the importance of cell proliferation and cell transformation with chemicals was shown in *in vitro* systems.[66] The multistep nature of the transformation process is becoming evident.[151] Morphologic alterations, including altered growth patterns and loss of topoinhibition (loss of contact inhibition), acquisition of ability of cells to grow in soft agar or without spreading on a solid surface (loss of anchorage dependence), and ability to grow as a neoplasm in appropriate intact animals (syngeneic or "immunologically crippled," eg, athymic nude mice) appear to be discrete or identifiable characteristics that can be isolated and studied separately. Another interesting use is in the identification and analysis of promoters and promotion. For example, saccharin, a possible promoter for urinary bladder cancer,[152] was shown to facilitate the transformation of C3H/10T½ cells with some chemical carcinogens.[151] Also, *in vitro* systems have been widely used in the analysis of the effects of phorbol esters in promotion.[97,98] Among other important contributions of *in vitro* systems are the comparisons between various animal and human cells with respect to (1) patterns of activation, (2)

types of adducts between carcinogens and DNA, and (3) DNA repair patterns.

In Vitro *Organ Culture*

An old approach that has recently been revived is organ culture, using either animal or human tissues. Surviving pieces from organs, such as trachea and bronchi, skin, esophagus, and urinary bladder are stabilized for a few days and then exposed to various procarcinogens and carcinogens. Both growth and differentiation can often be maintained for weeks or longer in appropriate mediums; and a variety of aspects of chemical carcinogenesis, including activation and its modulation, types of quantitation of adducts between carcinogens and DNA, DNA repair, and the appearance of focal hyperplasias, dysplasias, and even transformation are now being studied in detail. So far, cell transformation has only been found in animal preparations, since the human tissues have not been maintained for periods longer than several weeks. Comparisons between animal and human preparations with respect to many parameters have been fruitful.[49-51, 154]

Combined In Vivo *and* In Vitro *Models*

The past few years have seen the development of a few such systems with considerable success.[7] In general, animals are treated with appropriate carcinogens (eg, N-ethyl-N-nitrosourea [ENU]; dimethylnitrosamine [DMN] and so on) and tissues from kidney, brain, lung, fetus, and so on, have been placed in cell culture and followed for weeks or months. The stepwise acquisition of properties such as increased growth rates, growth in soft agar or methyl cellulose, and tumorigenicity on transfer back to animals offers a further approach to the sequential analysis of the development of cancer with chemicals.

Newer In Vivo *Models*

Ultimately, hypotheses developed from all the different approaches will have to be subjected to tests in animals and in selected human situations. Since the phenotype of a cell is determined by the environment as well as by its genetic makeup, the milieu in an intact organism may be required to test the relevance of a proposed mechanism to cancer. Very few of the many systems used in experimental studies on chemical carcinogenesis *in vivo* have evolved to the point where a stepwise analysis seems feasible. Skin, liver, and mammary gland of selected rodents are three such. In each of these systems, some patterns of cancer development do mimic to varying degrees the patterns seen in man.[7]

New Bioassays for Carcinogens and Promoters

The basic knowledge that has been generated during the past 20 years or so has spawned a large array of short-term tests, mostly *in vitro*, for potential carcinogens and more recently for promoters. These tests fall in general into two broad groups: those that use some correlate of DNA damage or chromosomal damage as end points and those that use cell transformation as an endpoint.[93,155-158] As with tissues *in vivo*, many carcinogens require metabolic conversion to active derivatives before they manifest their effects. This necessitates the inclusion of an appropriate activation system in all short tests for precarcinogens.

This development is an essential requirement for rapid monitoring of our environment and is leading to new insights into possible hazards in human occupational and other exposures.[159,160]

In addition, they are used increasingly as rapid assays for some more basic aspects of chemical carcinogenesis, such as patterns of activation in tissues, endogenous production of carcinogens or mutagens, variations in ability to metabolize carcinogens during the pathogenesis of cancer or other disease processes, and the analysis of active forms of carcinogens during metabolism, to name but a few of many examples.

Overview

It is evident from this brief discussion and from more extensive reviews[5,7] that cancer in many sites often arises as a stepwise multistep process. This conclusion can no longer be contested.

However, the models or systems available for study of the genesis, properties, options, and fates of each of such sequential cell populations as steps in the development of cancer and their roles, if any, in the evolution toward cancer are still not well developed. For the early changes, the skin and liver remain the best models. However, no model yet exists for the study of a possible sequence of events beginning with a persistent focal proliferation and ending in some aspect of malignant neoplasia. In almost every instance, the different steps that occur run together in a manner that does not enable the identification and analysis of any discrete link in this chain. In our view, the main reason for this problem is the "self-generating" nature of this apparently "environment-independent" segment of the carcinogenic process. Also, in every model used, the number of early pre-

neoplastic lesions is far larger, by a factor of 10^2–10^4, than the number of cancers ultimately seen. This is a serious limitation to the sequential analysis of cancer development. It would be highly desirable to develop a model in which the number of early, middle, and late lesions equals the number of cancers. Only in such a model could one be certain that one is studying the carcinogenic process. The available liver and skin models may allow this refinement even now.

Up to now, we have been content to act to a large degree primarily as *naturalists*, observing nature as it appears, with only a minimum of experimental manipulation of the process. While essential at the beginning of the study of any biologic phenomenon, such an approach cannot hope to elucidate in a factual way any process except the simplest and most elementary of biologic phenomena.

This lesson was clearly presented in the exciting developments in skin carcinogenesis in the 1930–1950 decades. During this period, the *experimental* or *in vivo manipulative* approach proved itself. Yet somehow, even today, this lesson has gone unheeded except in an occasional study of carcinogenesis in the liver, urinary bladder, mammary gland, and connective tissue.

It appears that increasing emphasis has been and is being placed on *in vitro* systems as the major hope. These have shown interesting and, in a few instances, exciting developments. Yet only in a few combined *in vivo* and *in vitro* approaches have they opened up new insights into the development of cancer as it occurs in an intact animal or human.[7] Although the study of transformation *in vitro* with chemicals is a legitimate endeavor in basic biologic science, there consistently appears a desire or a compulsion to relate the findings to the intact organism. Given the high reactivity of activated carcinogens with DNA and many other cellular constituents and the diversity in the products seen, given the extreme diversity of biologic effects of active promoters such as some of the phorbol esters, given the absence of the highly restrictive homeostatic system in the intact organism that limits severely what actually happens, and given the plasticity and adaptability of cells and cell systems when removed from the restrictions of the homeostatic forces *in vivo*, there exists a reasonable probability that any biologic phenomenon studied *in vitro* may not have a close relevance to the phenomena of the development of cancer *in vivo*. Therefore, a close interplay between *in vitro* and *in vivo* studies would appear to be an important prerequisite for a mechanistic analysis of carcinogenesis. For example, it is clearly evident that perhaps as much as 50% or 60% of the time taken for the development of epithelial cancer with chemicals involves altered or initiated cells that show no ability to escape from the rigid growth control in normal tissues. It is in this segment of the process that promotion by external agents or manipulations becomes evident. *In vitro*, this period of no autonomous growth is largely bypassed. How does one relate the *in vitro* to the *in vivo*?

Another aspect that needs some discussion is the need for systems that require *many different manipulations* if the multistep process of carcinogenesis is to be dissected into its component steps. This principle is elementary and yet seems not to have received due attention in studies of the carcinogenic process with chemicals. The more manipulations required to propel the system toward cancer, the more analyzable the model.

As already mentioned, if a model is designed or is being used for metabolic or for dynamic biologic approaches to the analysis of the sequence of changes, some way to obtain discrete cell populations synchronized with respect to the step involved must be developed. The newer cell sorters may be useful, despite the relatively small numbers of cells involved, if suitable markers can be identified. An alternative approach is to manipulate the experimental system so as to augment or amplify the cell population under study.[110]

A highly desirable property of a model is a testable hypothesis. By the latter is meant a proposal, based on a rational analysis, that can be subjected to a clear-cut experimental test. Unfortunately cancer, including carcinogenesis, has been the subject of enormous speculative activity for many decades. In the early years, when the scientific technology and knowledge were meager, few alternatives to speculation were present. Given the current state of scientific technology, realistic hypothesis testing, rather than grandiose speculation, would seem to be very appropriate.

The hypothesis testing is needed at different levels of biologic organization. Yet this testing seems to be hampered by the early acceptance of concepts based on minimal or no data. To cite but two examples: 1) The concept of loss of growth control dominates current views of initiation and promotion. Yet even a casual examination of its foundation is anything but convincing.[7] 2) A commonly expressed view is that cancer development with chemicals requires "multiple hits" by the carcinogen on the cells that might evolve into cancer.[161,162] Although several "rare events"[7] are probably necessary for the ultimate development of cancer, there is no clear-cut evidence

that these need to be or even can be induced by a carcinogen. For example, in the three instances in which a likely precursor population, the skin papilloma, was exposed to a carcinogen, no influence of the carcinogen on the development of cancer was observed in two.[163-165] While the paucity of experimental data does not allow any conclusion at this time, it would be premature to conclude that another "hit" by a carcinogen is influencing in a major way the evolution of a papilloma to a carcinoma. At least two other possibilities must be considered. The first, the most radical, would propose that the carcinogen gives *no* information to a cell that relates directly to cancer but merely induces a cell that can be selectively amplified by a promoting environment. The second, less radical, would propose that the postinitiation effect of carcinogens are largely, if not entirely, related to the promoting effects of the carcinogen, ie, in selectively encouraging the growth of the initiated cells by effects on the surrounding cells. Clearly, scientific analysis demands that such notions be entertained and tested. Without a more critical view of the possibilities, it is unlikely that novel approaches to the analysis of early steps in the carcinogenic process will be developed.

Despite these criticisms and limitations, it appears that cancer research is approaching a more critical view of each step in the process whereby cancer occurs with chemicals. Given the complexity of *any* single malignant neoplasm from any point of view—morphologic, biochemical, immunologic, biologic—it appears unlikely that the problem of the essential nature or natures of any malignant cell population can be solved by the exclusive emphasis on the cancer cell. It is likely that a historic approach, with emphasis on the stepwise sequence of cellular, biologic, biochemical, molecular, and immunologic changes leading to cancer, coupled with a focused study of cancer cell populations, would be more appropriate from the current perspective.

Summary and Perspectives

The past decade has seen a rapid expansion of our understanding of how some chemicals begin the process of the development of cancer. The increasing knowledge has included new insights about the requirement for metabolic activation, about the nature of the active molecules, about the enzymology of activation and the array of products generated, and about those that may be major ultimate carcinogens. We have learned much about the interactions of such derivatives with DNA and other cell constituents and about the possible toxic and other effects on cells. There is a general feeling that DNA damage is involved in cancer initiation in most instances.

The importance of the modulation of metabolic activation by enzyme induction with drugs, chemicals, diet, and hormones is appreciated generally. The enzymes involved, mainly in the microsomal mixed-function oxygenase system, can be induced by many environmental contaminants such as pesticides, herbicides, and polychlorinated biphenyls (PCBs). How this ubiquitous exposure may influence cancer development in humans is not known.

The repair of lesions in DNA is receiving increasing emphasis, especially since a defect in this process is associated with some genetic diseases with a high cancer risk. The majority of such studies are in relatively simple *in vitro* systems and are generating insights into what *might* happen. The study of what *does happen* remains a major challenge. This study may be aided by the development of ultrasensitive enzymatic radioimmunoassays for carcinogen–DNA adducts.

These basic studies have generated a host (over 100) of short-term assays for possible carcinogens, many of which reflect damage to DNA or chromosomes. Their prognostic utility as initial screens for potentially toxic environmental agents is being explored.

The past decade has also seen an increasing realization that many post-initiation phenomena, including promotion, are of major importance for cancer development. In general, promoters do not interact with DNA and select an initiated cell population to undergo focal proliferation. Alterations in diet, hormones, drugs, and xenobiotic agents have major influences in chemical carcinogenesis through effects on promotion and subsequent steps in this multistep process.

The increasing awareness of the reversibility of many of the precancerous steps in cancer development is fostering an interest in the interruption of the process through the use of vitamin A and its analogs and of other dietary components.

Despite the major advances, few new insights into the essential nature of the carcinogen-altered cells during initiation have been generated. Although cell proliferation is required for initiation with chemicals, its exact role and the biologic nature of the initiated cells remain largely unknown. Even though knowledge about the further evolution to cancer could generate new and important ways to interrupt the development of cancer and so prevent the disease, the study of these later phases of chemical carcinogenesis lags far behind that of the very early biochemical events.

The studies to date are consistent with the thesis that human tissues in general react very much like animal tissues in their handling of chemical carcinogens. Also, very recent studies have shown that human cells can be transformed *in vitro* with various chemical carcinogens and that the increasing body of knowledge about *in vitro* carcinogenesis is applicable in principle to human cells.

However, a major uncertainty in this field in general concerns the role of chemical carcinogens, either of exogenous or endogenous origin, in the etiology and pathogenesis of some of the major forms of cancer in humans. There is a general agreement that cigarette smoking and ultraviolet light are two major factors in the genesis of several important types of cancers. Also, there is incontrovertible evidence that many chemicals to which humans are exposed in the workplace or its environs cause cancer in several organ systems. However, the relative quantitative importance of these in the majority of cancers not related to smoking is unclear.[166,167] The determination of the roles of chemicals in carcinogenesis, although no doubt difficult, will almost certainly have a major impact on our approaches to the prevention of cancer in coming decades.

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DETERMINING "SAFE" LEVELS OF EXPOSURE: SAFETY FACTORS OR MATHEMATICAL MODELS?

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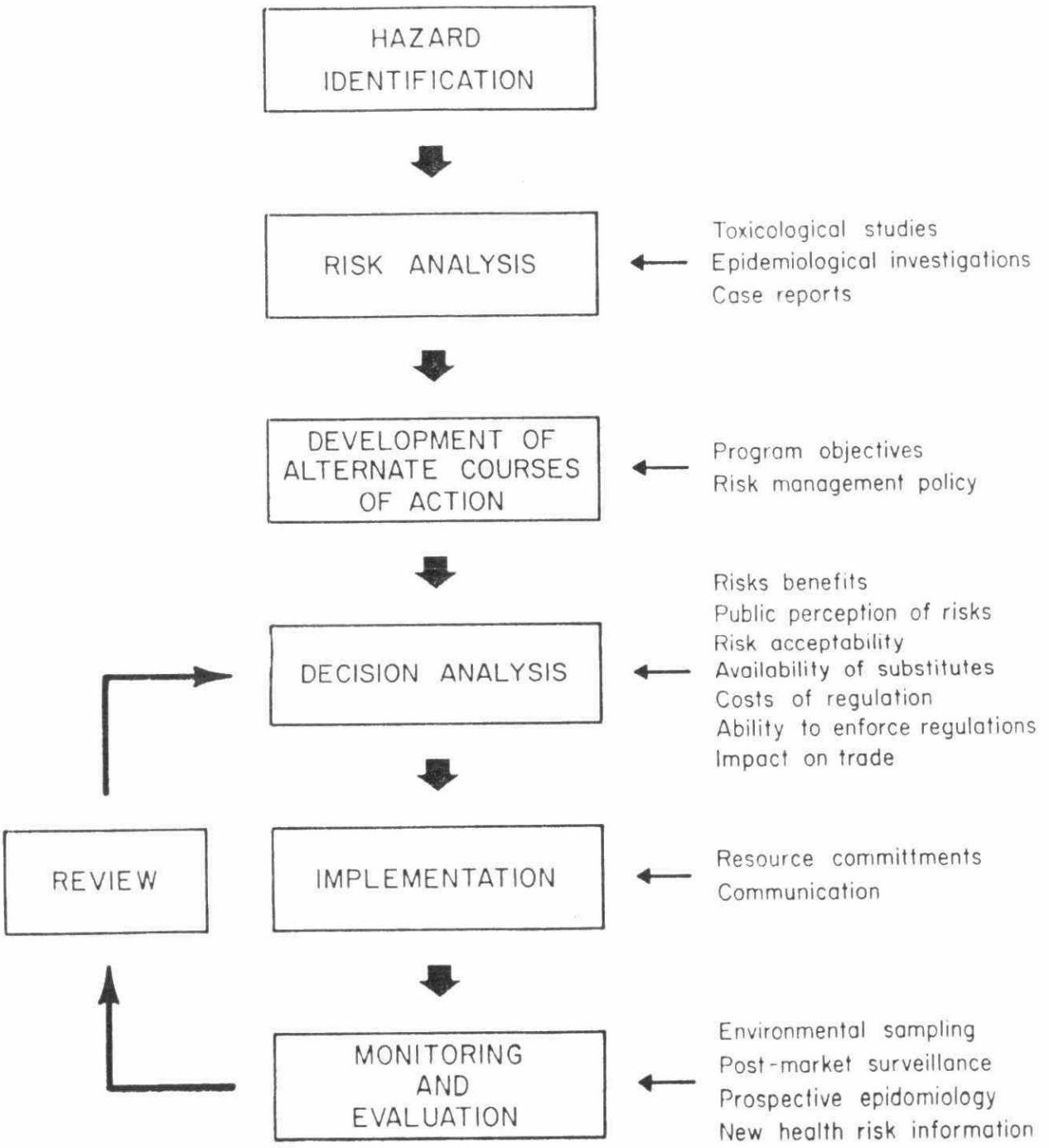
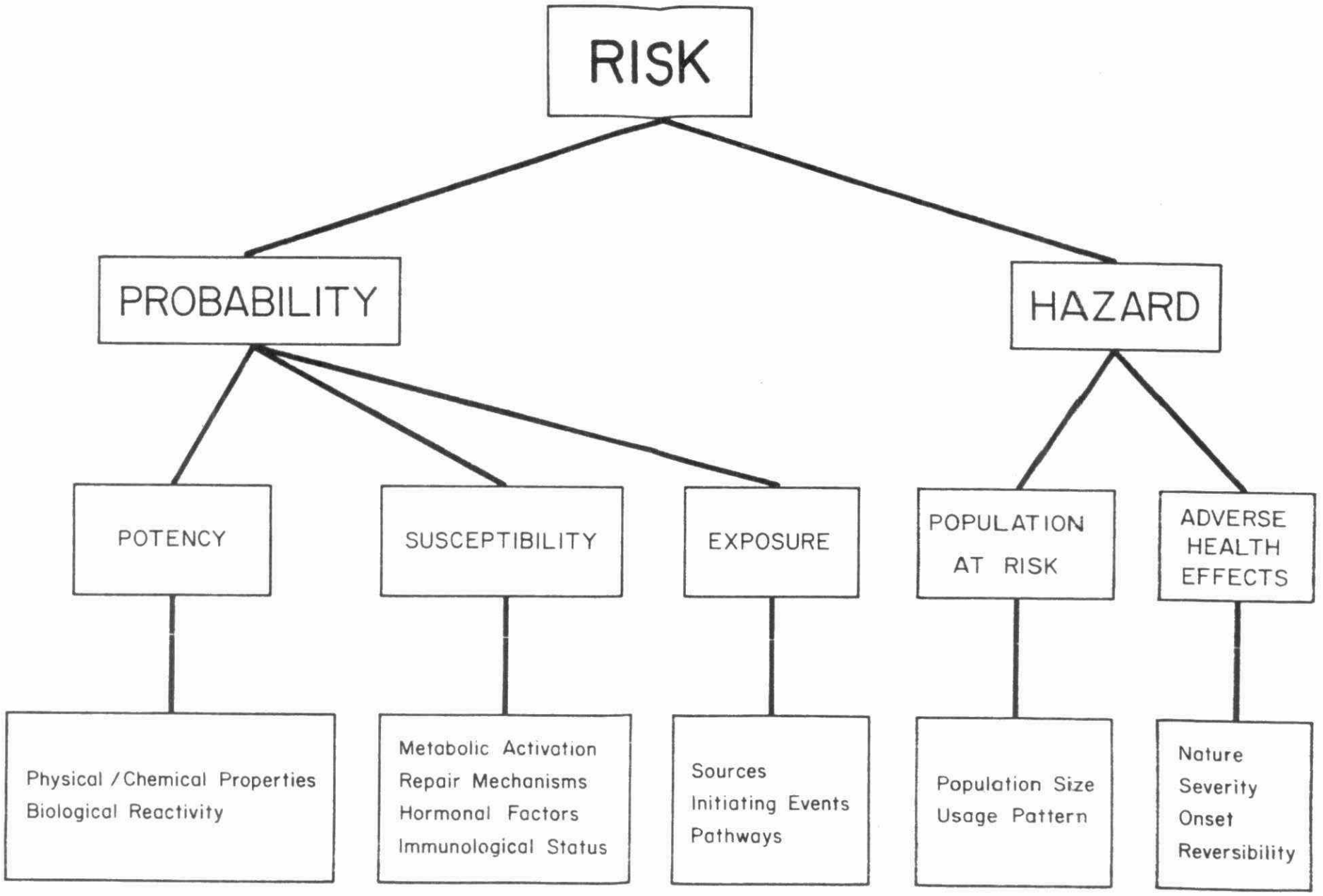

HAZARD IDENTIFICATION
RISK ANALYSIS
Toxicological studies
Epidemiological investigations
Case reports
DEVELOPMENT OF ALTERNATE COURSES OF ACTION
Program objectives
Risk management policy
DECISION ANALYSIS
Risks benefits
Public perception of risks
Risk acceptability
Availability of substitutes
Costs of regulation
Ability to enforce regulations
Impact on trade
IMPLEMENTATION
Resource committments
Communication
MONITORING AND EVALUATION
Environmental sampling
Post-market surveillance
Prospective epidomiology
New health risk information
REVIEW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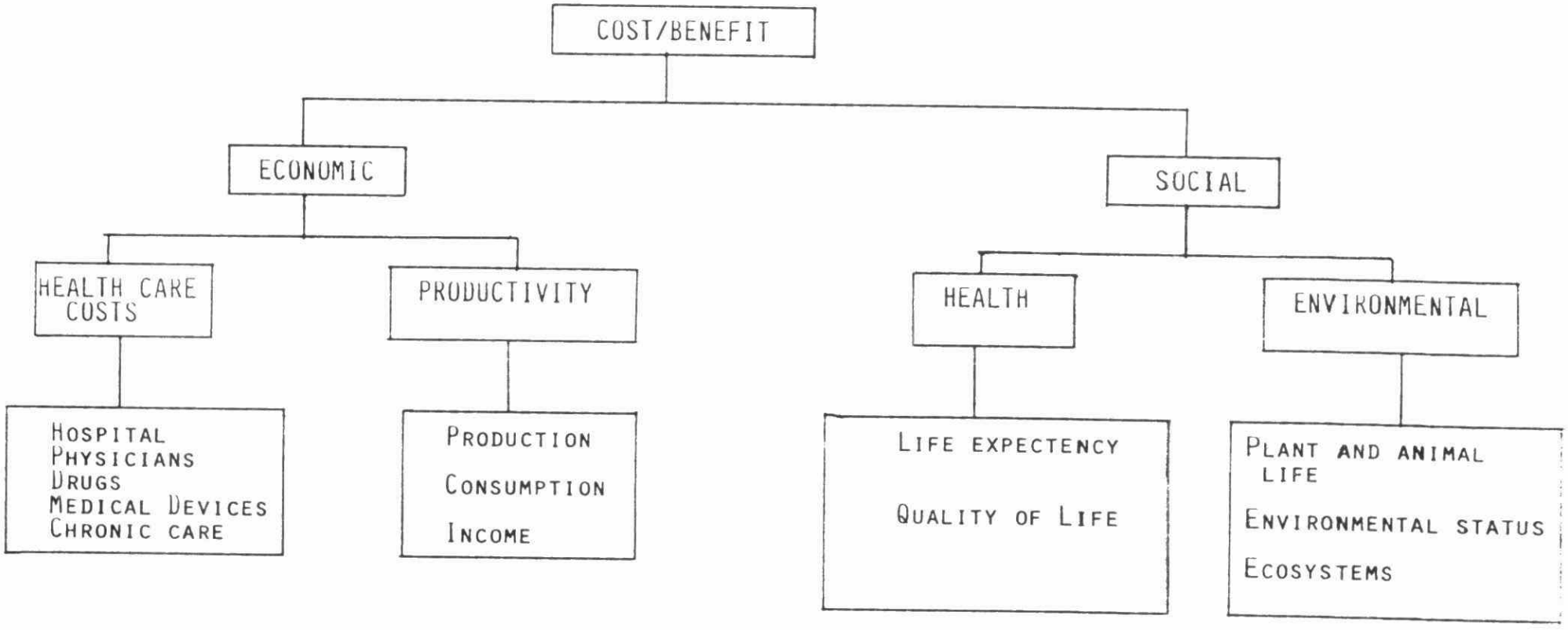
COST/BENEFIT
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HEALTH
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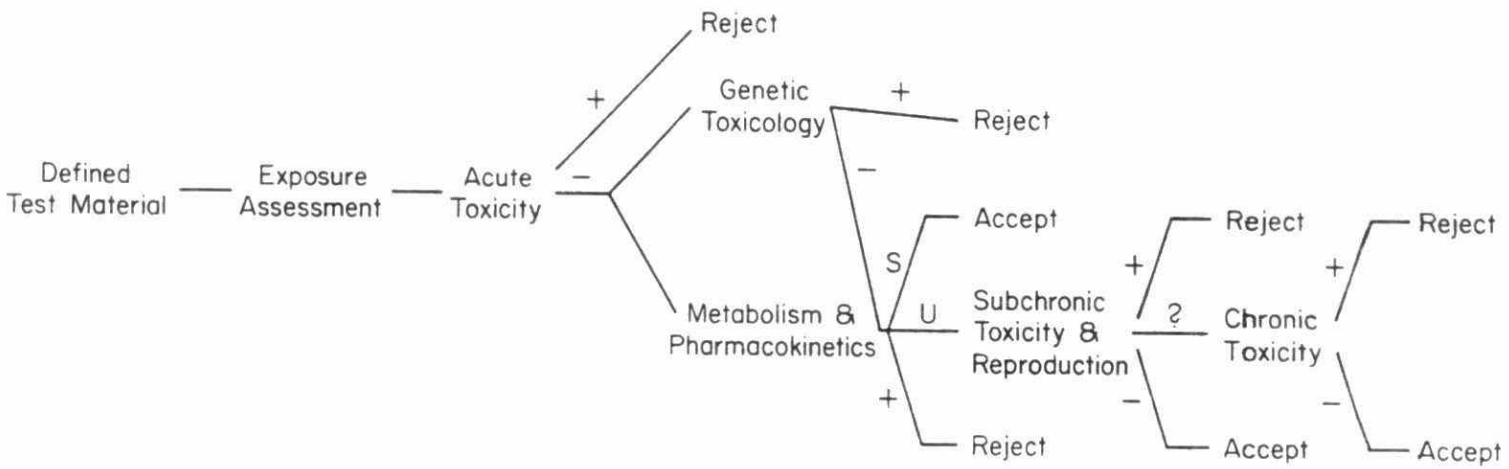
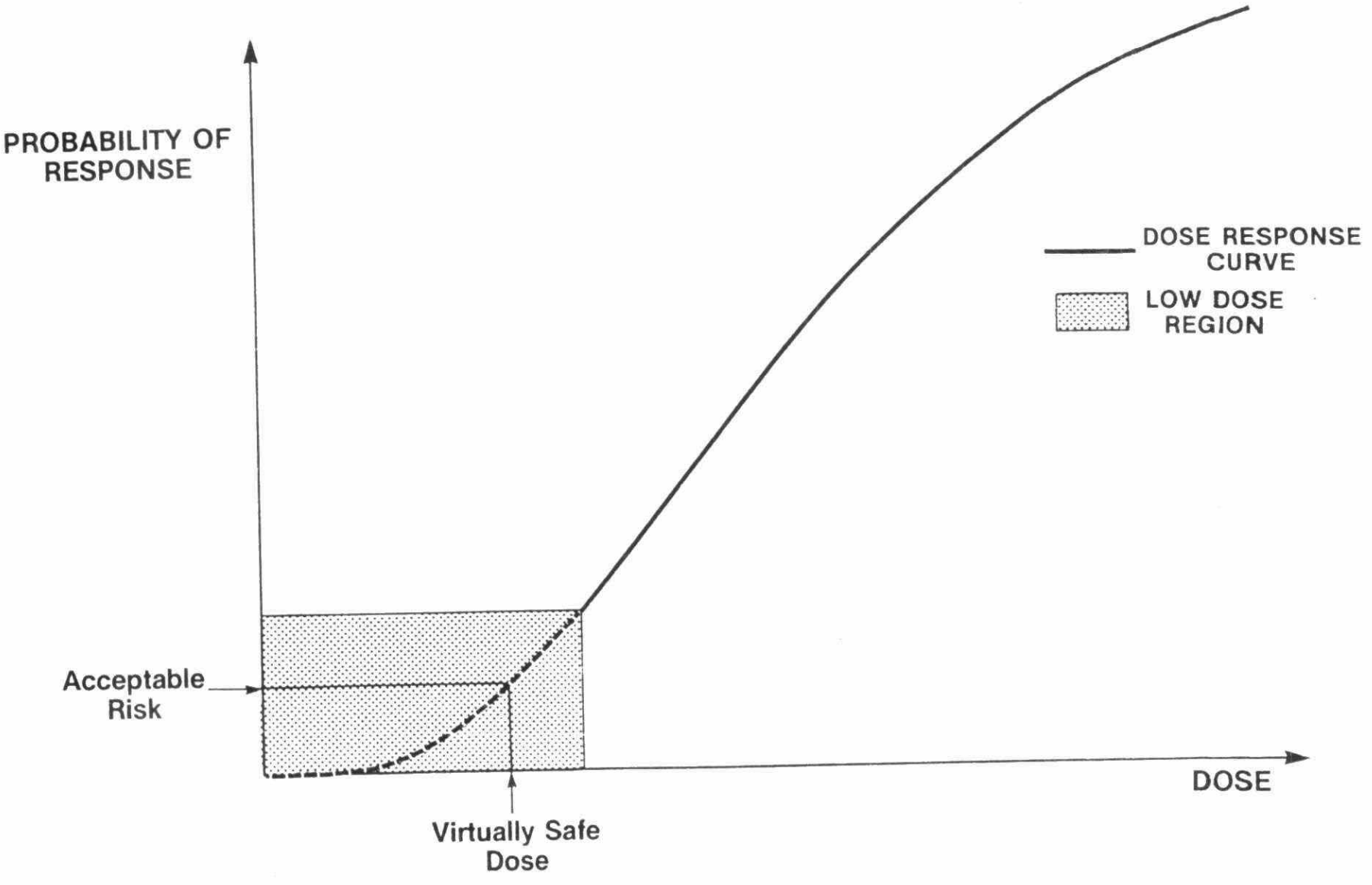
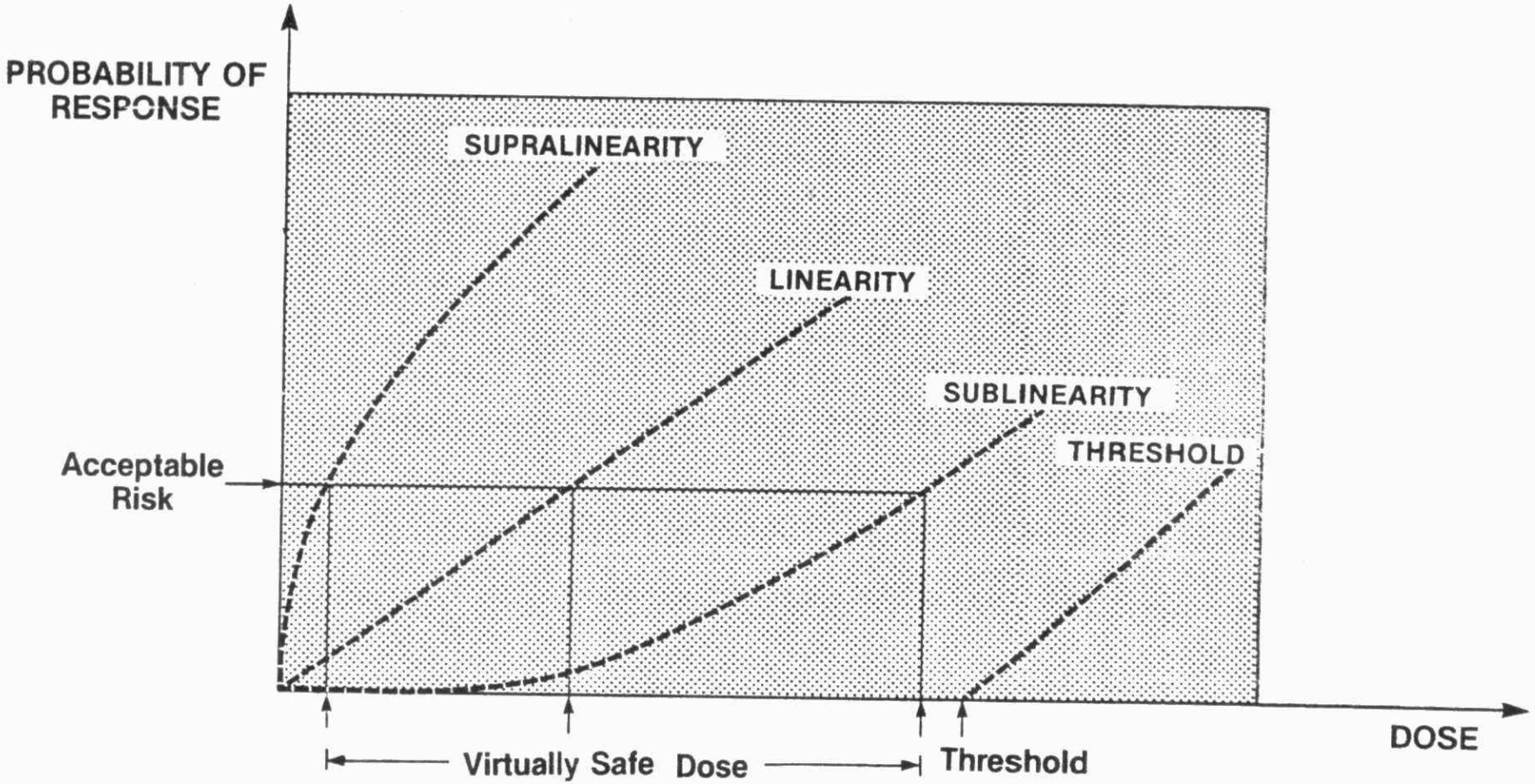
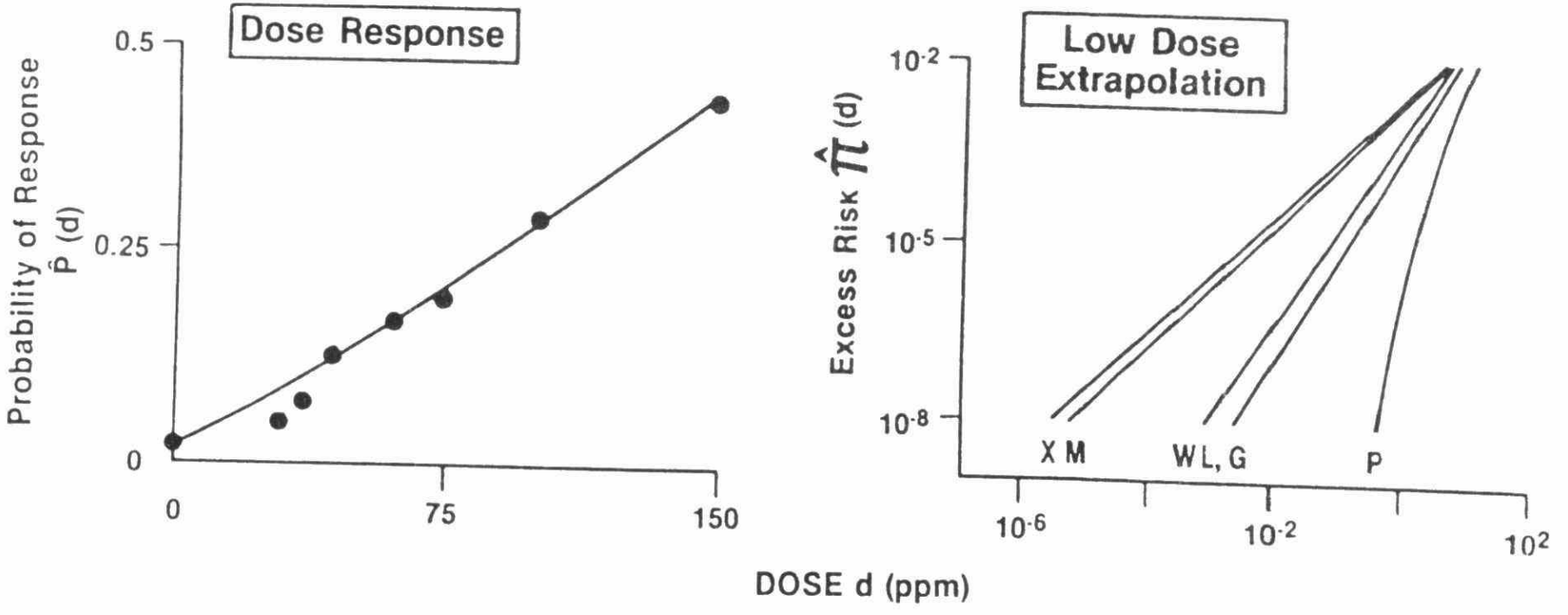
HOSPITAL
PHYSICIANS
DRUGS
MEDICAL DEVICES
CHRONIC CARE
PRODUCTION
CONSUMPTION
INCOME
LIFE EXPECTENCY
QUALITY OF LIFE
PLANT AND ANIMAL LIFE
ENVIRONMENTAL STATUS
ECOSYSTEMS



+ = presents socially unacceptable risk
− = does not present a socially unacceptable risk
S = metabolites known and safe
U = metabolites unknown or of doubtful safety
? = decision requires more evidence

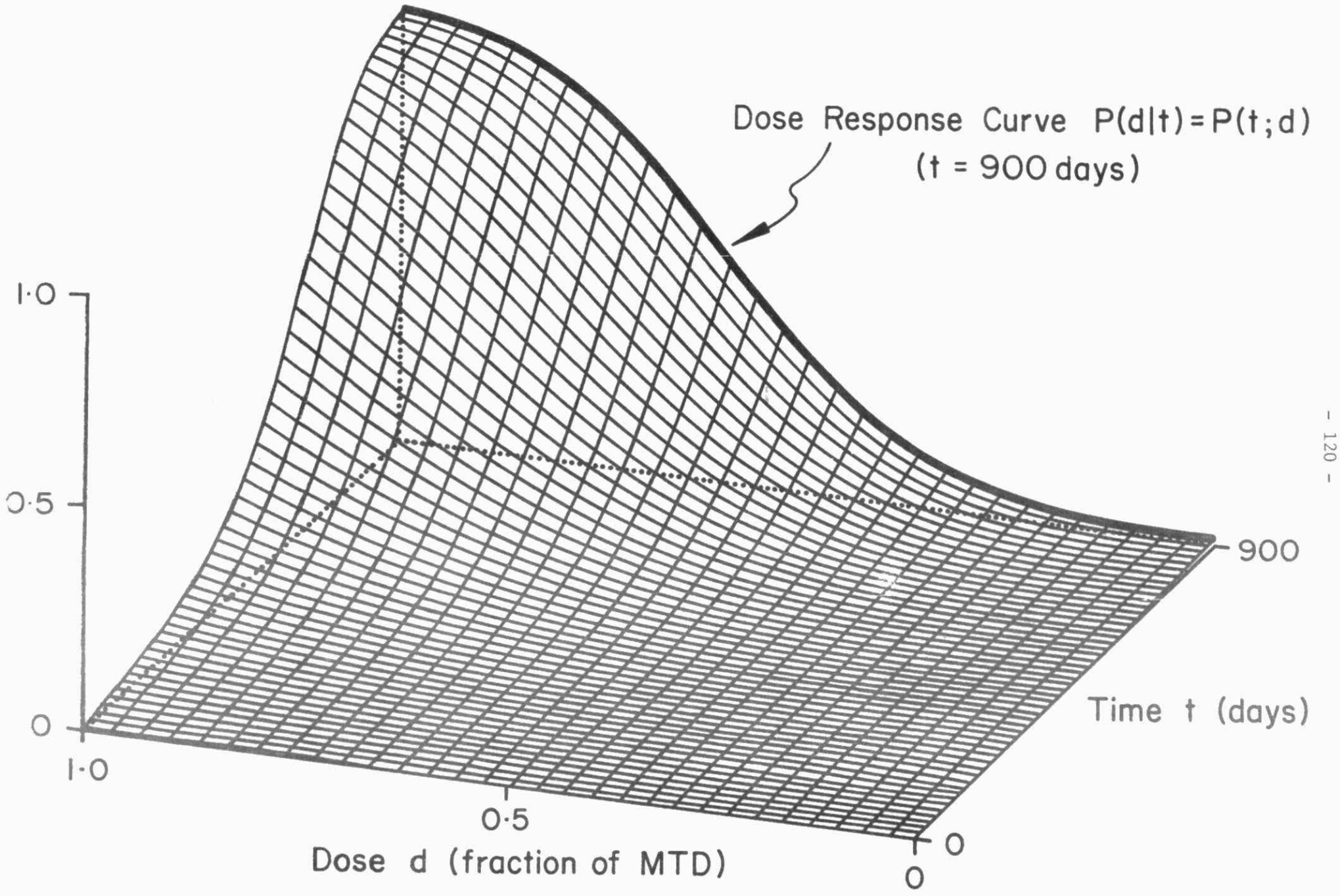

PROBABILITY OF RESPONSE
DOSE RESPONSE CURVE
LOW DOSE REGION
Acceptable Risk
DOSE
Virtually Safe Dose

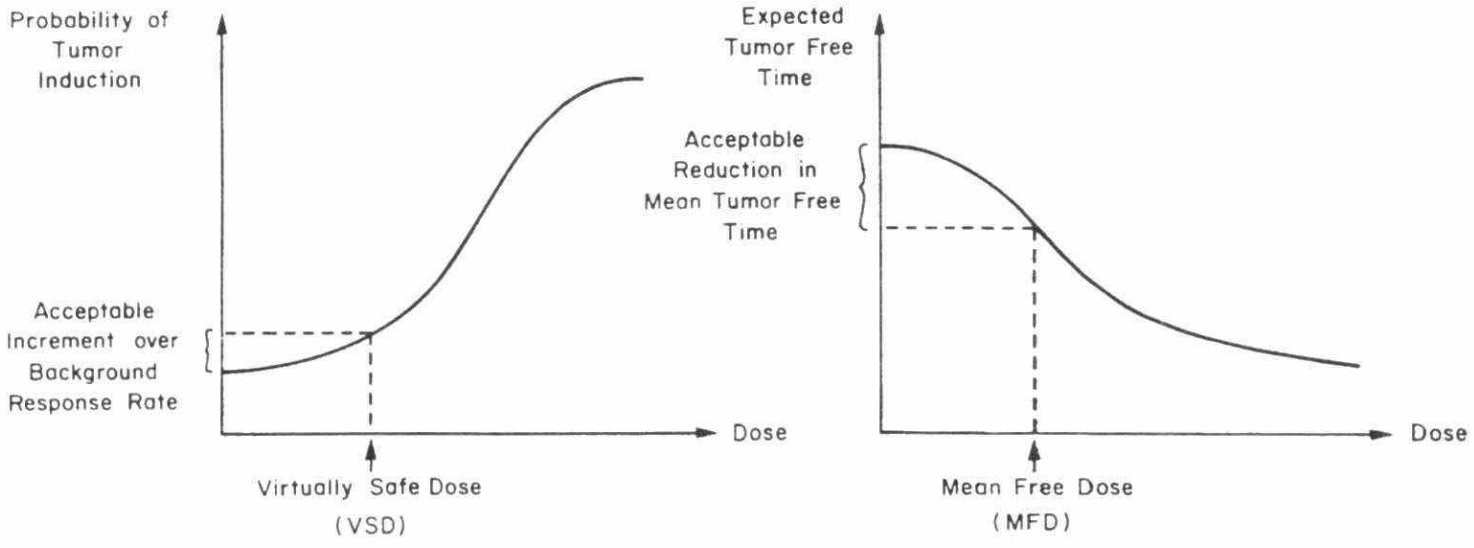

PROBABILITY OF RESPONSE
SUPRALINEARITY
LINEARITY
SUBLINEARITY
THRESHOLD
Acceptable Risk
Virtually Safe Dose
Threshold
DOSE



X — Linear Extrapolation
M — Multi-Stage Model
W — Weibull Model

L — Logit Model
G — Gamma Multi-Hit Model
P — Probit Model


Dose Response Curve P(d|t) = P(t;d)
(t = 900 days)
1·0
0·5
0
1·0
0·5
Dose d (fraction of MTD)
0
0
900
Time t (days)


Probability of Tumor Induction
Acceptable Increment over Background Response Rate
Dose
Virtually Safe Dose (VSD)
Expected Tumor Free Time
Acceptable Reduction in Mean Tumor Free Time
Dose
Mean Free Dose (MFD)

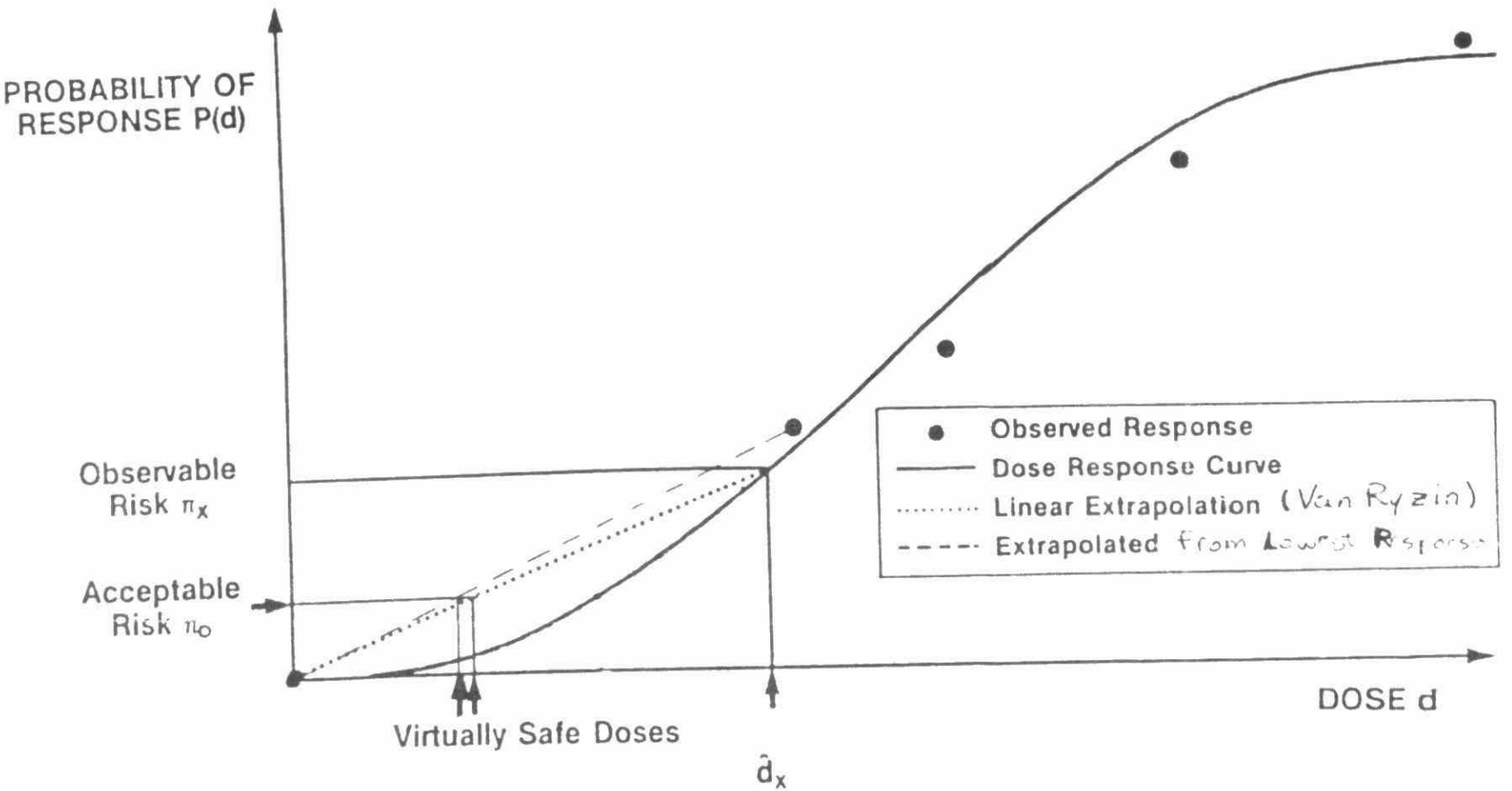

PROBABILITY OF RESPONSE P(d)
Observable Risk π_x
Acceptable Risk π_o
Observed Response
Dose Response Curve
Linear Extrapolation (Van Ryzin)
Extrapolated from Lowest Response
DOSE d
Virtually Safe Doses
$\hat{d}_x$

Figure 4.

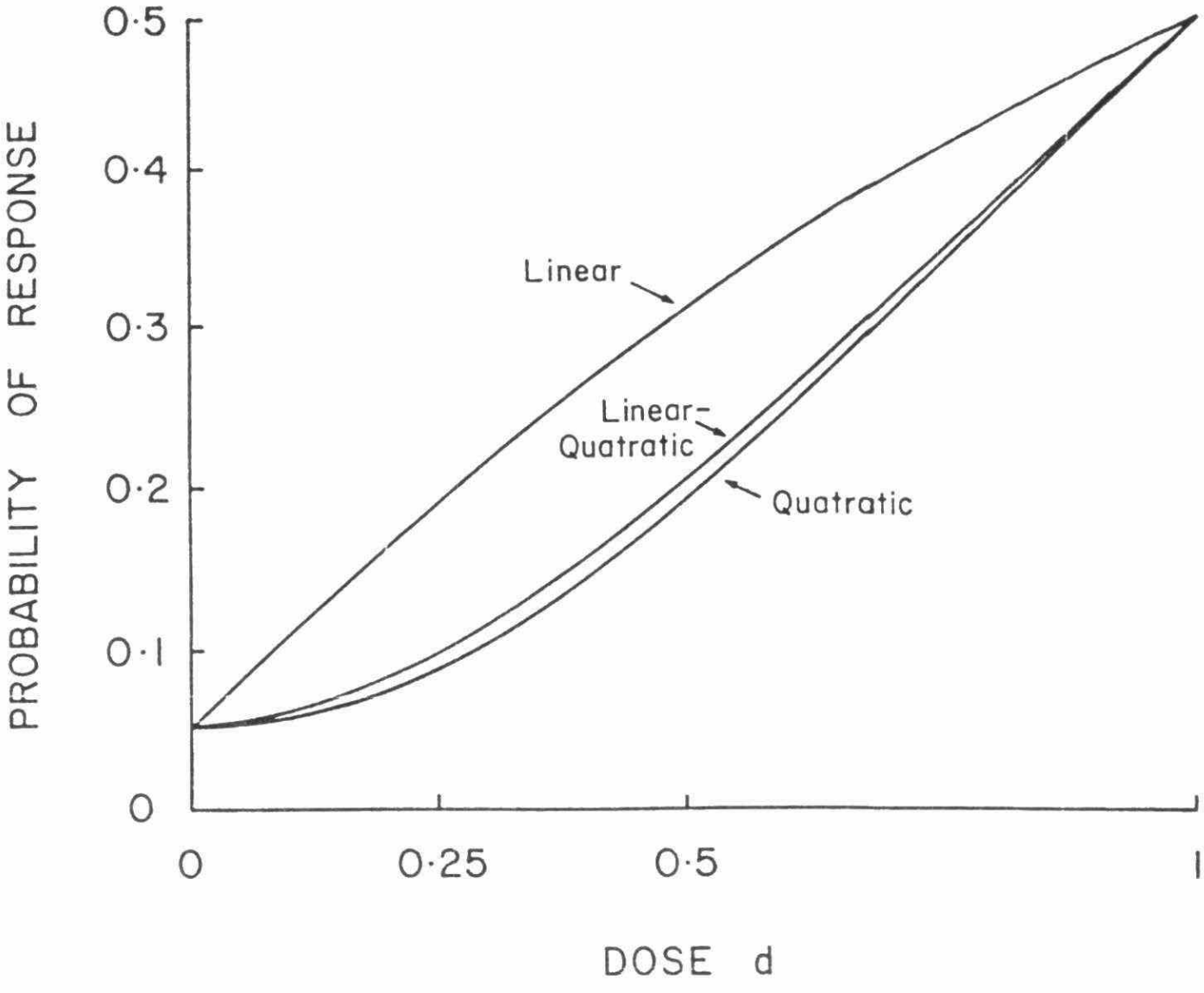

PROBABILITY OF RESPONSE
0·5
0·4
0·3
0·2
0·1
0
Linear
Linear-
Quatratic
Quatratic
0
0·25
0·5
1
DOSE d

Figure 5.

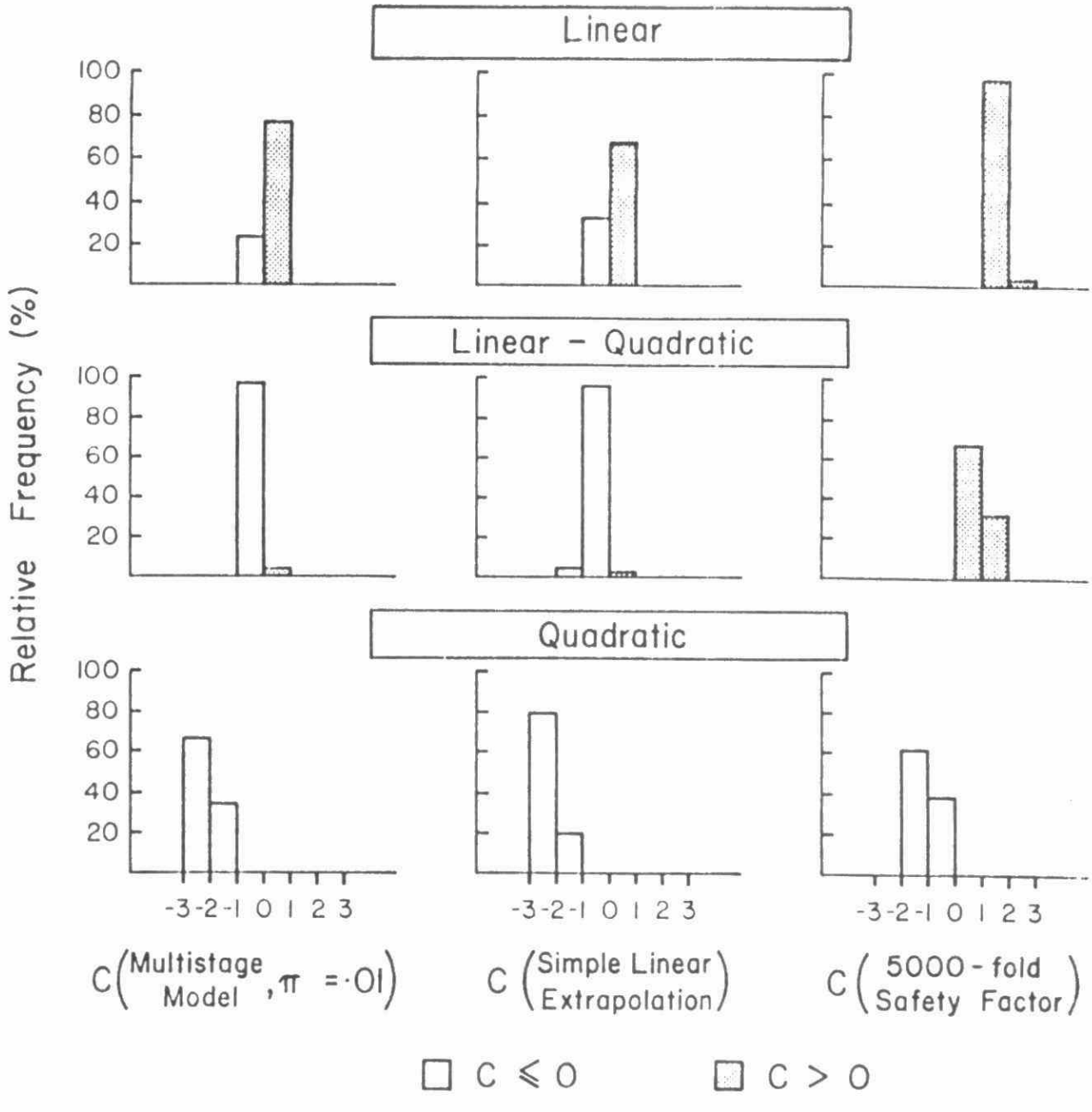

Linear
Linear - Quadratic
Quadratic
Relative Frequency (%)
100
80
60
40
20
-3 -2 -1 0 1 2 3
C(Multistage Model, π = ·01)
C(Simple Linear Extrapolation)
C(5000 - fold Safety Factor)
□ C ≤ 0
□ C > 0

Figure 7.

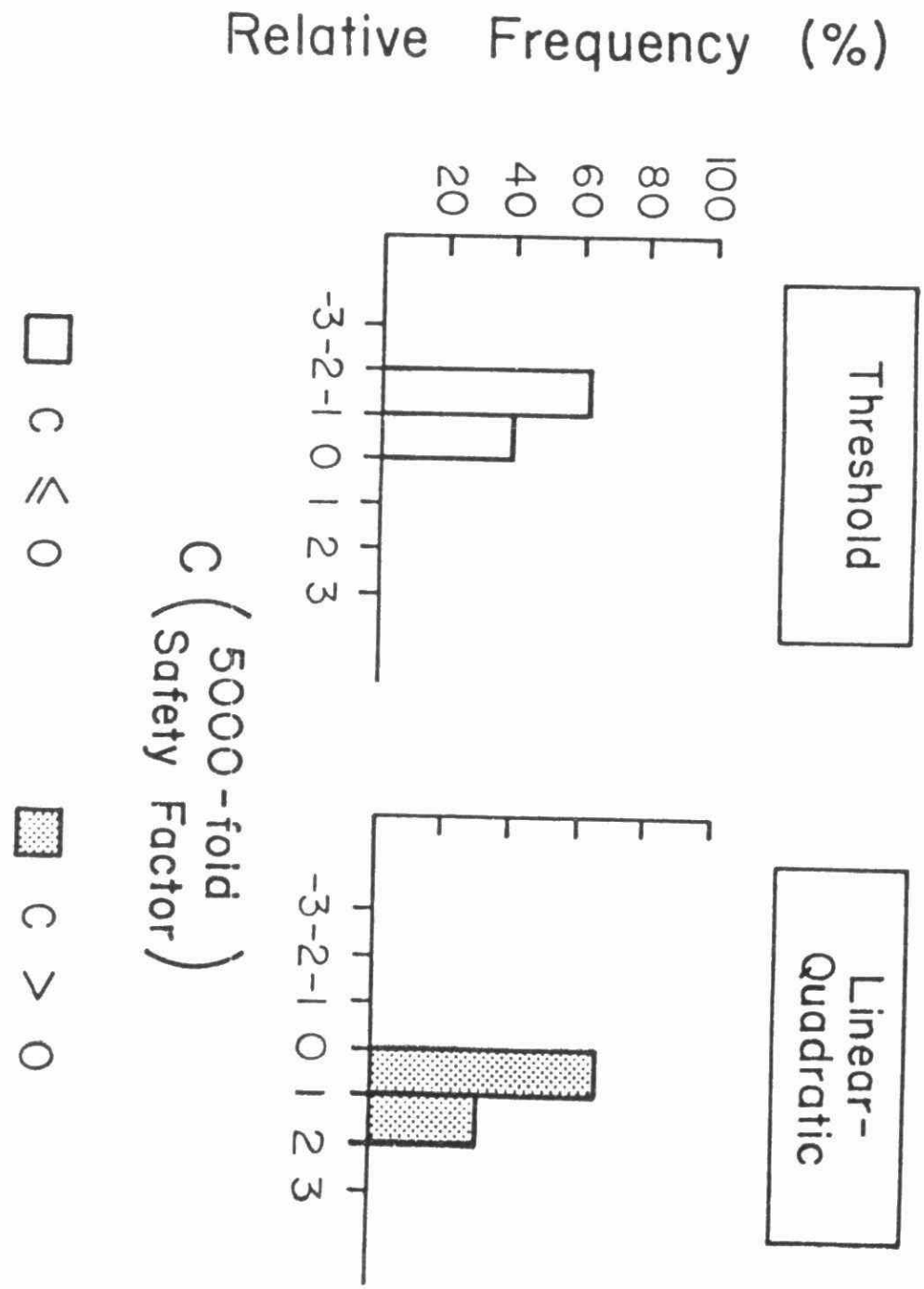

Relative Frequency (%)
100
80
60
40
20
-3-2-1 0 1 2 3
Threshold
Linear-Quadratic
C (5000-fold Safety Factor)
C ≤ 0
C > 0

Figure 8.

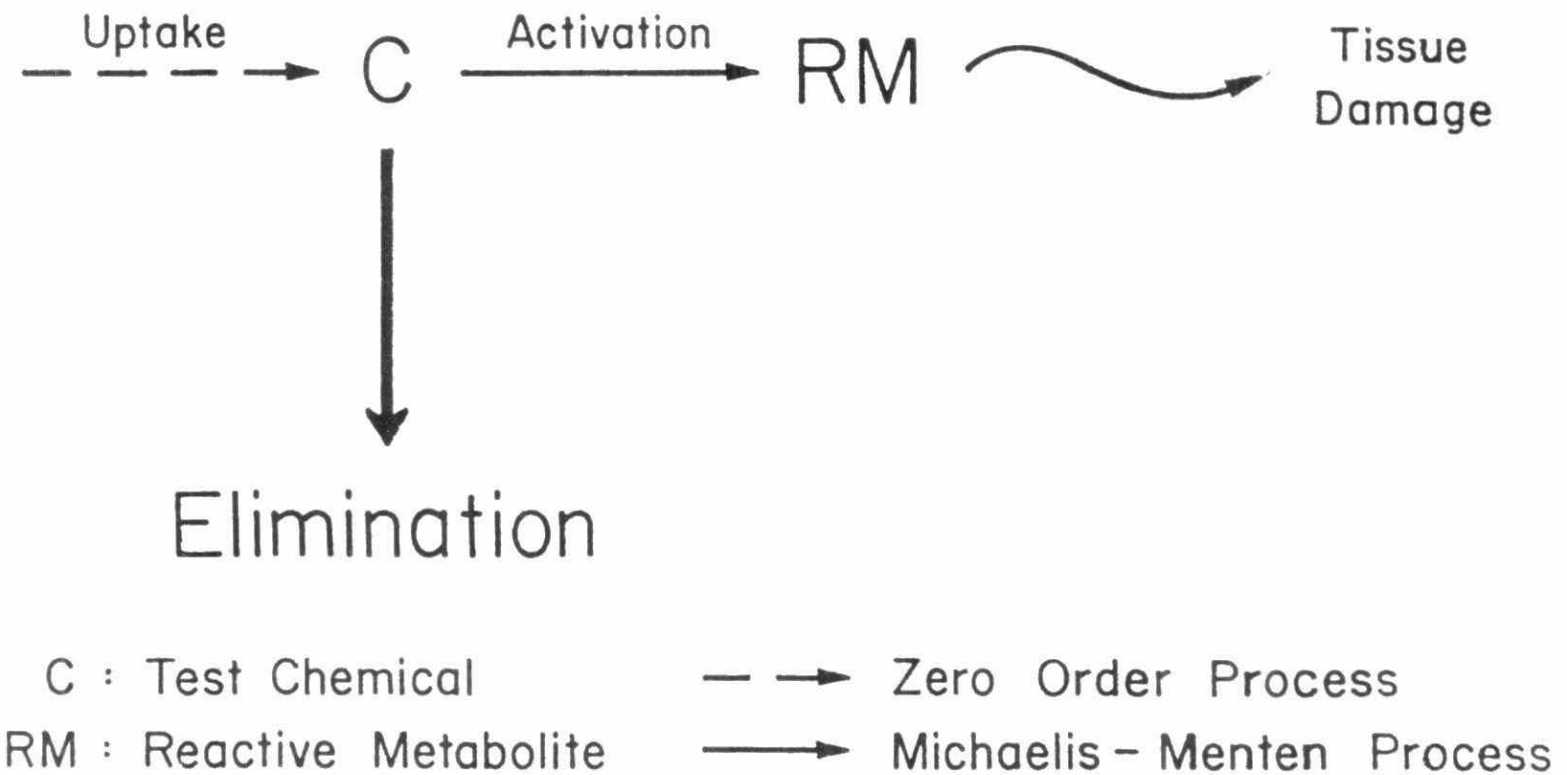

Uptake
C
Activation
RM
Tissue
Damage
Elimination
C : Test Chemical
RM : Reactive Metabolite
Zero Order Process
Michaelis - Menten Process

Figure 9.

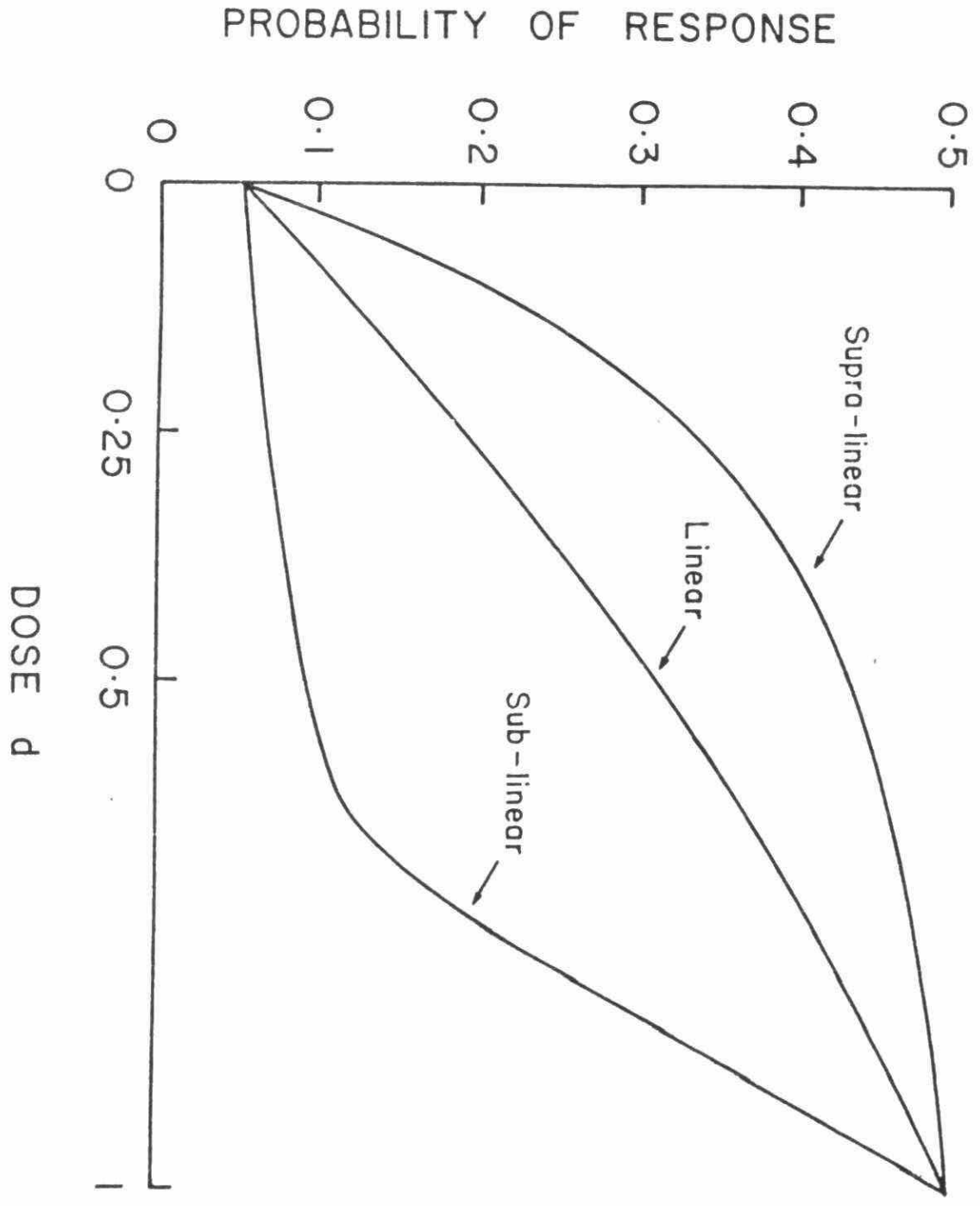

PROBABILITY OF RESPONSE
0
0·1
0·2
0·3
0·4
0·5
0
0·25
0·5
1
DOSE d
Supra-linear
Linear
Sub-linear

Figure 10.

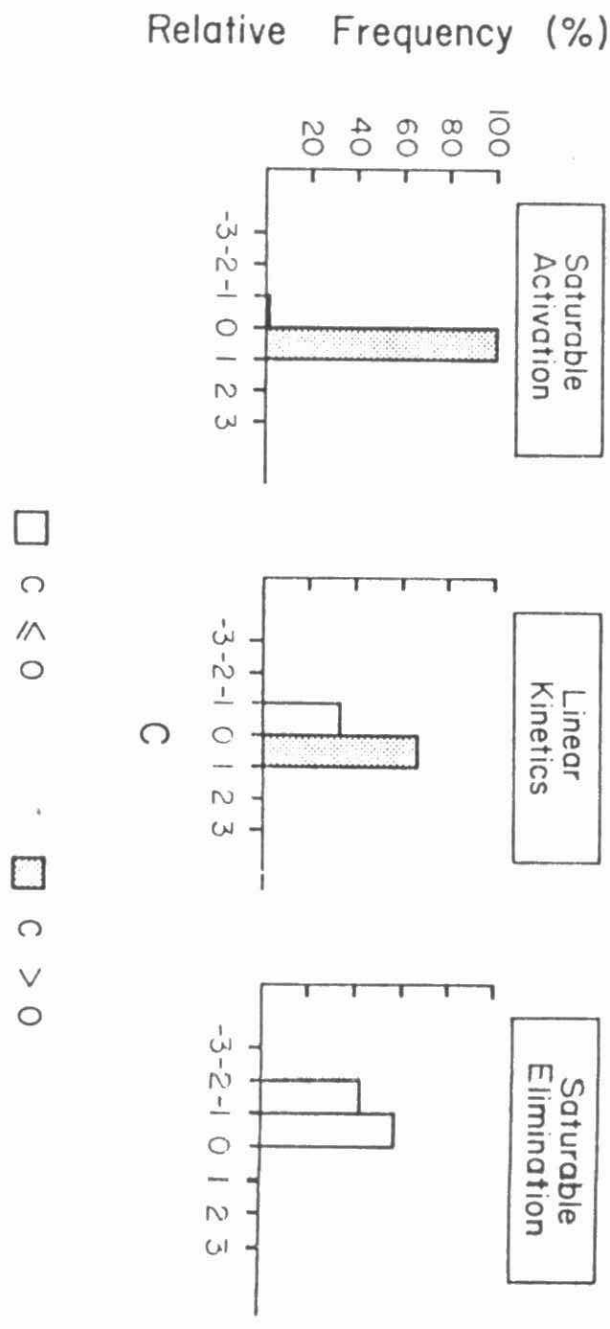

Relative Frequency (%)
100
80
60
40
20
-3-2-1 0 1 2 3
Saturable Activation
Linear Kinetics
Saturable Elimination
C
C ≤ 0
C > 0

Figure 11.

TCDD CARCINOGENICITY DATA

STUDY	SPECIES	SEX	ENDPOINT	RESPONSE				HIGH DOSE
				Control	Low	Mid	High	
Kociba[a]	Rat	M	Squamous cell carcinoma	0/77	2/44	1/49	9/44	.1 µg/kg/day
		F	Hepatocellular carcinoma or neoplastic nodule	16/86	8/50	27/50	33/47	.1 µg/kg/day
NCI[b]	Rat	M		0/74	0/50	0/50	3/50	.5 µg/kg/wk
		F		5/75	1/49	3/50	14/49	.5 µg/kg/wk
NCI[b]	Mouse	M	Hepatocellular carcinoma or adenoma	15/73	12/49	13/49	27/50	.5 µg/kg/wk
		F		3/73	6/50	6/48	11/47	2 µg/kg/wk

a Doses in ratio 0 : 1 : 10 : 100.

b Doses in ratio 0 : 1 : 5 : 25.

EXTRAPOLATION OF TCDD CARCINOGENICITY DATA

STUDY	SPECIES	SEX	ENDPOINT	POTENCY (EXCESS RISK PER UG/KG/DAY)	VSD (10^{-5} RISK) (PG/KG/DAY)
KOCIBA (S-ANALYSIS)	RAT	M	SQUAMOUS CELL CARCINOMA (NASAL TURBINATE, HARD PALATE OR TONGUE)	45	0.2
		F	HEPATOCELLULAR CARCINOMA OR NEOPLASTIC NODULE	43	0.2
NCI		M		0.7	14
		F		3.5	3
NCI	MOUSE	M	HEPATOCELLULAR CARCINOMA OR ADENOMA	35	0.3
		F		14	0.7

FISH
(Lake Ontario)

20 ppt TCDD

113 g fish/wk

60 kg person

5.4 PG/KG - B.W./D

VSD AT 10^{-5} LIFETIME RISK:

0.2 - 14 PG/KG/DAY

CARCINOGENIC POTENCY

$$P = 7 - \log_{10} ED_{25}$$

Carcinogen Class	Examples	Regulatory Options
I	Tris	Ban
II	2-Naphthylamine	
III	Chloroform	
IV	NTA	
V	Saccharin	Limited use, labelling, public education

DETERMINING "SAFE" LEVELS OF EXPOSURE: SAFETY FACTORS OR MATHEMATICAL MODELS?

The object of regulatory toxicology is to determine "safe" levels of human exposure to toxicants present in the environment. In this article, we compare the traditional safety factor approach to more recent mathematical modelling techniques, outlining the underlying assumptions and statistical properties of each procedure. Several linear extrapolation procedures are examined in detail using computer simulation, along with the impact of nonlinear kinetics on the extrapolation process.

1. Introduction

Centuries ago, Paracelsus (1493-1541) noted that "All things are poisons, for there is nothing without poisonous qualities. It is only the dose which makes a thing a poison." This simple observation has two important implications. First, overexposure to almost any substance can pose serious health risks. Second, the level of risk depends on the level of exposure. The fundamental problem of toxicology is thus to determine safe levels of exposure to toxicants present in the environment.

Although conceptually straightforward, the safety evaluation process is fraught with uncertainty (Miller et al, 1983). Scientific uncertainties prevail with respect to disease etiology, dose response relationships and the predictive value of toxicological data. Regulatory uncertainties hinge upon the notion of safety itself: is absolute safety an achievable goal or are small risks coupled with offsetting benefits acceptable? The increasing frequency with which environmental regulations are being challenged in the courts also bears witness to the legal uncertainties inherent in the statutes under which safety evaluation is mandated.

There are currently four major approaches to environmental health risk assessment (OTA, 1981). While epidemiological studies of human populations can provide direct evidence of adverse health effects in man, such studies are often clouded by uncertainty with respect to exposure levels and the possibility of confounding risk factors which may mask or otherwise obscure any effects of the putative causal agent. Perhaps more importantly, epidemiology necessarily involves an assessment of disease causality after the fact, and is thus inconsistent with a preventative regulatory philosophy. All of these problems can be overcome through the use of carefully conducted toxicological studies using animal models for man, but at the expense of obtaining only indirect evidence of potential health hazards to the human population. Because many physiological and biochemical processes are common to a number of mammalian species, however, toxicological studies have come to form the cornerstone of much of today's health risk assessment practice. Supplementary data is also provided by short-term in vitro tests designed to detect mutational and other events thought to be part of the process of carcinogenesis (NRC, 1983a) and sometimes by comparisons of the physical and chemical properties of the agent of interest with those of known toxicants.

A wide spectrum of toxicological tests have been devised to assess a variety of possible adverse health effects. Recently, several attempts have been made to systematize the application of these tests in a logical and orderly fashion. One of the most notable is the decision tree proposed by the Food Safety Council (FSC, 1982). The preliminary stages of this approach involve a determination of physical and chemical properties of the agent in question and an assessment of potential human exposure levels. Following the completion of

acute toxicity studies, a decision is made as to whether the compound is unsafe or to proceed simultaneously with a specific battery of short term tests for genotoxicity as well as studies on metabolism and pharmacokinetics. While evidence of serious genotoxic effects would likely halt further testing, additional tests might be considered unnecessary if no mutagenic effects were observed and if the substance was readily metabolized into residual levels of normal body constituents. Otherwise, subchronic and reproductive studies would be undertaken, followed by chronic toxicity studies in cases where human exposure is expected to be high or where the previous tests could not provide adequate assurances of safety.

Once the available data has been carefully evaluated, the regulatory toxicologist faces the difficult problem of establishing a safe level of exposure for man. From the regulatory point of view, this raises the issue of absolute versus virtual safety. The manner in which this question has been addressed in the past may be determined by examining the positions taken in legal statutes concerned with environmental health risk management. The recent review by the National Academy of Sciences (NRC, 1983b) revealed that both zero-risk and "de minimus" risk positions have been taken in different situations. A well-known example of the former case is the 1958 Delaney amendment to the U.S. Food and Drug Act of 1954 (see Coulston, 1979, for a detailed discussion). With respect to direct food additives, this piece of legislation specifically states that "no compound shown to cause cancer in man or animal may be added to food". Examples of the latter case include the setting of tolerances for food contaminants such as aflatoxin under Section 406 of the same (Miller, 1983) and the establishment of water quality criteria under the Clean Water Act (EPA, 1980).

In this paper, we examine the available procedures for establishing acceptable levels of human exposure to carcinogenic substances in the environment based on the results of long-term bioassay studies conducted in small rodents (Bickis & Krewski, 1984). We begin with an overview of the use of safety factors and mathematical models for this purpose (section 2). Several procedures for linear extrapolation are considered in section 3, and their propeties studied using computer simulation techniques. These results are also compared with those based on the use of a 5,000-fold safety factor. The impact of nonlinear kinetics in the metabolic activation process on risk estimation is examined in section 4. A summary of our findings and recommendations is presented in section 5.

2. Safety Factors and Mathematical Models

2.1 Safety Factors

The traditional approach to setting acceptable levels of human exposure to environmental toxicants has been to apply a suitable safety or uncertainty factor to that dose level at which no adverse effects were observed in toxicological studies. This approach seems to have been formalized first by Lehman & Fitzhugh (1954). In a detailed review of this procedure, Dourson & Stara (1983) note that a 100-fold safety factor has often been applied for general toxic effects other than cancer. This safety factor allows for a 10-fold variation in susceptibility between animal and man as well as a 10-fold variation in individual susceptibily within the human population. In the

absence of a no-effect level, an additional factor of 5 or so may be incorporated. A safety factor of 5,000 has been proposed for certain kinds of carcinogenic effects (Weil, 1972; Truhant, 1980), with the additional factor of 10 being due to the severity of the response.

In applying the safety factor technique, the underlying assumptions should be clearly understood. Foremost among these is the presumed existence of a threshold dose below which all individuals in the population at large will be free of risk. The safety factor approach also makes no distinction between laboratory response rates of 0/10 and 0/1000, thereby ignoring sample size. Finally, this approach takes little cognizance of the shape of the dose response curve, with steep and shallow slopes being treated more or less alike.

2.2 Mathematical Models

A more recent approach to establishing safe levels of exposure involves the application of mathematical modelling techniques to bioassay data in an attempt to determine a virtually safe dose or VSD corresponding to some suitably low level of risk (Figure 1). Formally, the VSD is defined by that dose d_0 for which

$$\pi = P(d_0) - P(0),$$

where $P(d)$ denotes the probability of a response at dose d and π is the acceptable risk level.

The shape of the dose response curve in the low dose region can have an appreciable effect on the VSD (Figure 2). Dose response curves approaching zero at a faster than linear or supralinear rate are theoretically possible under certain constructs, such as in a heterogeneous population comprised of a high proportion of susceptible individuals (Hoel, 1984). From the practical point of view, however, such a disproportionate increase in risk with increasing dose

seems somewhat questionable. It is now widely accepted that dose response relationships for carcinogenic agents will be at worst linear in the low dose region. For compounds which appear to act via the same mechanism by which spontaneously occuring lesions arise, moreover, it can be shown quite generally that low dose linearity will in fact obtain (Crump et al, 1976). Other observations consistent with such behaviour for compounds acting through direct interaction with genetic material include the linearity of many mutagenicity data sets at subtoxic doses and the linear relation between the formation of DNA adducts and low doses of several alkylating agents (Hoel et al, 1983). For compounds which appear to induce tumours due to secondary effects as the result of metabolic overload at high doses, on the other hand, even sulinear or threshold effects cannot be ruled out. Because of the statistical difficulties in determining thresholds however, most models have dispensed with the threshold concept (Brown, 1976).

(Insert Figures 1&2 about here)

The many mathematical models proposed to date vary in their degree of biological sophistication (Munro & Krewski, 1981; Le Cam, 1982). Simple tolerance distribution models such as the probit, logit and Weibull postulate the existence of a certain distribution of tolerances within the population, with the absence of a population threshold reflected in the fact that the minimum of the individual tolerances is allowed to be zero. Stochastic models such as the multi-hit or multi-stage assume that a positive response is the result of the random occurence of a specified number of hits at the target tissue or the completion of a certain number of stages in the response process.

Other approaches involve a compartments and the rate of tumour occurence as a function of dose within the critical compartment (Hartly et al., 1981).

As an example, consider the estimates of excess risk over background in the low dose region shown in Figure 3 for 2-AAF induced liver tumours in mice exposed for up to 24 months (Littlefield et al., 1979) based on the models noted above. These estimates are based on standard maximum likelihood procedures under the assumption that spontaneous and treatment induced lesions occur independently (Krewski & Van Ryzin, 1981). The corresponding estimates of the VSD at risks of 10^{-5} or lower span several orders of magnitude, with the multi-stage, Weibull, multi-hit, logit and probit models leading to progressively higher estimates. Although the magnitude of the differences between models will depend to some extent on the experimental data, the relative ranking of the corresponding VSD's may be expected to be similar to that obtained here whenever the observed data exhibit some degree of upward curvature. Unfortunately, statistical goodness-of-fit tests are not sufficiently powerful so as to be able to distinguish between two plausible models (Crump, 1981), leaving the choice of model uncertain.

(Insert Figure 3 about here)

In contrast to the use of simple quantal data considered in the previous example, several methods for exploiting information on time to tumour occurence are also available (Kalbfleisch et al., 1983). Application of these procedures is however hampered by the difficulties in obtaining accurate information on time to tumour occurence. Perhaps more importantly, recent investigations have shown that such procedures will not resolve the uncertainty as to the shape of the dose response curve in the low dose region even if precise information on time to tumour were available (Krewski et al., 1983).

3. Linear Extrapolation Procedures

In the preceding section, it was suggested that the linear hypothesis may represent a realistic position with respect to the low dose behaviour of some carcinogenic agents. For others displaying sublinearity or even threshold effects, moreover, linear extrapolation will provide an upper limit on the actual risk in the low dose region. In this section, we consider some simple methods of linear extrapolation and contrast the properties of these procedures with the use of a 5,000-fold safety factor applied to the lowest effect level observed in animal tests.

The proposed procedures are summarized in Figure 4. The first approach to linear extrapolation is due to Van Ryzin (1980), and involves extrapolating linearly from some point near the lower end of the fitted dose-response curve. A second approach is to extrapolate linearly from the lowest dose at which the observed response exceeds that in the control group. A slight modification of this simple linear extrapolation procedure is to smooth out any inversions in the observed dose-response curve using isotonic regression (Barlow et al., 1972), and then extrapolate linearly from the lowest dose above control. The final procedure we consider is the application of a 5,000 fold safety factor to the lowest dose at which the response is significantly ($p<.05$ in Fisher's exact test) above that in the control group.

(Insert Figure 4 about here)

Computer simulation was used to evaluate the performance of these four procedures. This involved generating 1000 sets of bioassay data in accordance with the two-stage models

$$P(d) = 1 - \exp\{-(\alpha_0+\alpha_1 d+\alpha_2 d^2)\}$$

shown in Figure 5 using an experimental

design with 50 animals at doses of d=0,¼,½ and 1 times the maximum tolerated dose. Each of the curves was constrained so as result in response probabilities of 5% and 50% at d=0 and 1 respectively. The linear model is linear at low doses as well as throughout most of the dose range. The linear-quadratic model is linear at low doses and exhibits some degree of upward curvature even at higher doses. The pure quadratic model is similar to the linear-quadratic model in terms of overall shape, but is sublinear in the low dose region.

(Insert Figure 5 about here)

The advantage of using simulated as compared to actual bioassay data is that the underlying model from which data arose is known, and provides a yardstick with which to guage the performance of the different extrapolation procedures. Our comparisons were based on the measure

$$C = \log_{10} (\hat{d}_0/d_0),$$

where d_0 denotes the estimate of the VSD d_0 at a risk of 10^{-6}. This criterion provides a measure of relative error on an order of magnitude scale. C=2, for example, would correspond to an overestimate of two orders of magnitude or a factor of 100. C=-1, on the other hand, would indicate the VSD had been underestimated by a factor of 10. By analyzing each of the three dose response models in Figure 5, we were able to obtain an estimate of the distribution of the errors C for each of the three linear extrapolation procedures considered as well as the safety factor approach.

The error distributions for the model based linear extrapolation procedure starting from excess risk levels of π_x=0.001, 0.01 and 0.1 are shown in Figure 6. Since taking π_x=0.001 resulted in several estimates which were between 10 and 100 times above the true VSD when the underlying dose response curve was linear, this procedure was less desirable than that based on π_x=0.01 or 0.1.

Although there was little to choose between the latter two values under the linear-quadratic model, π_X=0.01 was less conservative than π_X=0.1 in the pure quadratic case. (The consistent underestimation of the VSD under the quadratic model is a reflection of the low dose linearity imposed by the extrapolation procedure in the presence if a sublinear dose response curve.) Thus, we selected π_X=0.01 for further evaluation.

(Insert Figure 6 about here)

The error distributions for this procedure are compared to those based on simple linear extrapolation and the application of the 5000-fold safety factor (Figure 7). (The isotonic regression procedure yielded results which were essentially the same as those shown for simple linear extrapolation and are thus omitted.) While little difference was noted between the model-based and simple linear extrapolation procedures, the latter has the advantage of ease of computation—analysis of a single data set could in fact be performed in minutes without the aid of even a pocket calculator. When the underlying dose response curve is linear at low doses, these results also indicate that linear extrapolation is preferable to the safety factor approach because of the latter's consistent overestimation of the VSD by up to two orders of magnitude.

(Insert Figure 7 about here)

It should be emphasized that the superiority of the linear extrapolation over the safety factor approach has been demonstrated only when the dose response curve is truly linear at low doses. To illustrate the superiority of the safety factor approach in another situation, suppose that a threshold were introducted into the linear-quadratic model at d=0.001. Although this change would have an imperceptible effect on response rates above this dose, the safety factor would now underestimate the threshold dose by as much as it had overestimated the VSD under the original linear-quadratic model (Figure 8).

(Insert Figure 8 about here)

4. Pharmacokinetic Models for Metabolic Activation

In the preceding section, we evaluated the performance of several extrapolation procedures, using the administered dose as the dose metameter for extrapolation. Many compounds, however, require some form of metabolic activation in order to be effective (O'Flaherty, 1981). In this section, we therefore consider some simple pharmacokinetic models for metabolic activation, and explore the effects of nonlinear kinetics on the simple linear extrapolation procedure introduced in section 3.

Consider the metabolic activation scheme depicted in Figure 9. In this case, the concentration of the agent in the compartment into which the compound is taken up is assumed to be proportional to the administered dose, as might be expected with inhalation exposure. Once absorbed into the body, the compound may be either eliminated or converted to a reactive metabolite responsible for neoplastic tissue change. In this model, both elimination and activation are allowed to be saturable following Michaelis-Menten kinetics. The general relationship between the administered dose d and the dose of the reactive metabolite d* delivered to the target tissue is derived in the Appendix. For simplicity, we will assume in what follows that the probability of tumour induction is a linear function of the delivered dose (Hoel et al., 1983).

(Insert Figure 9 about here)

Under linear kinetics, the probability of tumour induction will be described by the linear model discussed in section 3. Under saturable activation, the dose-response curve may exhibit a distinct levelling out at higher doses, whereas the reverse effect may occure under saturable elimination (Figure 10).

(The values of the kinetic constants underlying these three cases are given in the Appendix.)

(Insert Figure 10 about here)

The performance of the simple linear extrapolation procedure introduced in section 3 based on simulation of these three situations is summarized in Figure 11. Although all three curves are linear in the low dose region, the procedure seems to be somewhat conservative in the case of saturable elimination. The reason for this is that the steepness of the dose response curve under saturable elimination results in frequent extrapolation from the maximum tolerated dose.

(Insert Figure 11 about here)

5. Summary and Conclusions

The fundamental problem of toxicology is to determine safe levels of human exposure to environmental toxicants based on the results of toxicological experiments. The solution to this problem depends to a great extent on the shape of the dose response curve in the low dose region, with the linear and threshold hypotheses representing perhaps the two plausible extremes. Since both possibilities will generally be consistent with data in the observable response range, the choice between them must be made on the basis of supplementary biological information concerning possible mechanisms of action. While the possibility of sublinearity lies between these two extremes, the chief difficulty with this concept in practice is that the appropriate degree of sublinearity cannot be readily determined on the basis of experimental data.

When low dose linearity is indicated, some form of linear extrapolation seems appropriate. Three possibilities were considered here. The first involved linear extrapolation from some point on the fitted curve where the excess risk is still in the observable range. Our limited simulation results

suggest that the 1% excess risk point may not be unreasonable in this regard. The second involves linear extrapolation from the first experimental point at which the response rate is above that in control. The third method proceeds in an analogous fashion after using isotonic regression to smooth out any revisions in the observed dose-response curve.

Under the conditions studied, each of these three linear extrapolation procedures was found to perform similarly. In the case of low dose linearity, the VSD was generally estimable to within a factor of 10 or so. In sublinear or threshold cases, however, linear extrapolation was found to underestimate the VSD by factors of up to 1000 or more. Conversely, the use of a 5000-fold safety factor applied to the lowest dose at which a statistically significant increase above control was observed was found to seriously overestimate the VSD in the case of low dose linearity, but was found to be superior to linear extrapolation in other cases. Again, the most suitable procedure cannot be selected on the basis of the observed data, although guidance in this regard may be obtained through careful consideration of all of the available biological data in other test systems.

In conclusion, we feel that the use of both linear extrapolation and safety factors represent viable approaches to establishing safe levels of exposure with the most suitable of these two methods depending on the biological acceptability of the linear no-threshold hypothesis. Refinements to the simple linear extrapolation procedure favored here may be developed so as to incorporate confidence limits and interspecies adjustment factors, although given the uncertainties in these latter two areas (Crump & Howe, 1983; Hogan & Hoel, 1983), the procedure may be useful as it stands at least as a first approximation.

Appendix: Pharmacokinetic Models for Metabolic Activation

In this appendix, we describe a simple pharmacokinetic model for metabolic activation of the test compound. For simplicity, details will be presented only for the case of inhalation exposure involving a constant concentration of the test chemical in the atmosphere. Assuming zeroth order kinetics, the rate of uptake is then simply $K_0 = pd$ $(0 < p \leq 1)$, where d denotes the concentration of the agent under study in the atmosphere.

Consider the kinetic model shown in Figure 9. Once absorbed, the test compound C may either be eliminated or activated to a reactive metabolite RM responsible for tissue damage. Both of these processes are allowed to follow Michaelis-Menten kinetics with respective maximum rates M_e and M_a and "first order" rate coefficients k_e and k_a at low doses.

This simple model is described by the system of nonlinear differential equations

$$\frac{d[C]}{dt} = K_0 - \frac{k_a M_a [C]}{M_a + k_a [C]} - \frac{k_e M_e [C]}{M_e + k_e [C]} \qquad (A.1)$$

and

$$\frac{d[RM]}{dt} = \frac{k_a M_a [C]}{M_a + k_a [C]} \qquad (A.2)$$

where [C] and [RM] denote the respective concentrations of the chemical absorbed and reactive metabolite formed by time t. Under the steady state condition $d[C]/dt = 0$, (A.1) may be written as the quadratic equation

$$a[C]^2 + b[C] + c = 0, \qquad (A.3)$$

where

$$a = k_a k_e (K_0 - M_a - M_e), \tag{A.4}$$

$$b = k_0 (M_a k_e + M_e k_a) - M_a M_e (k_a + k_e) \text{ and} \tag{A.5}$$

$$c = k_0 M_a M_e. \tag{A.6}$$

For the system to be stable, the rate of uptake of the test compound must be less than the maximum total rate of absorption and elimination, i.e., $K_0 < M_a + M_e$. Under this condition, $a < 0$ and (A.3) is a parabola opening downward. Since $c > 0$, (A.3) has one positive and one negative root, the former being

$$[C]^* = \frac{-b - (b^2 - 4ac)^{1/2}}{2a} \tag{A.7}$$

It now follows from (A.2) that

$$[RM]^* = \frac{k_a M_a [C]^*}{M_a + k_a [C]^*} \quad t \tag{A.8}$$

which gives the steady state concentration $[RM]^*$ of the reactive metabolite as a function of the atmospheric dose d at any time t.

It is of interest to note that $[RM]^* \to \gamma K_0$ as $K_0 \to 0$, where $\gamma = k_a/(k_a + k_e)$ is the fraction of the absorbed dose which is activated at low doses. As $k_0 \to M_a + M_e$, on the other hand, $[RM]^* \to k_e M_a^2/(k_e M_a^2 + k_a M_e^2)$. This is the limiting slope as the system becomes unstable.

Variation of the kinetic constants governing absorption, elimination and activation can lead to a spectrum of curves relating the delivered dose to the target tissue $d^* = [RM]^*$ to the target tissue to the adminstered dose d (Table 1). Similar effects are also observed under the linear dose response model

$$P(d) = 1 - \exp\{-(a_0 + a_1 d^*)\} \tag{A.9}$$

where $d^* = f(d)$ with f defined through (A.7) and (A.8) (see Figure 11 in the text).

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Table 1. Kinetic Constants Leading to Concave, Linear and Convex Relationships Between the Delivered and Administered Dose

($K_o = 1$, $p = 1$)

Shape of Dose-Response Curve	Kinetic Constant[a]			
	k_a	M_a	k_e	M_e
Concave	1	0.2	1	10
Linear	1	∞	1	∞
Convex	0.1	10	1	0.75

[a] Normalized so as to give f(d) = d at d=0 and 1.

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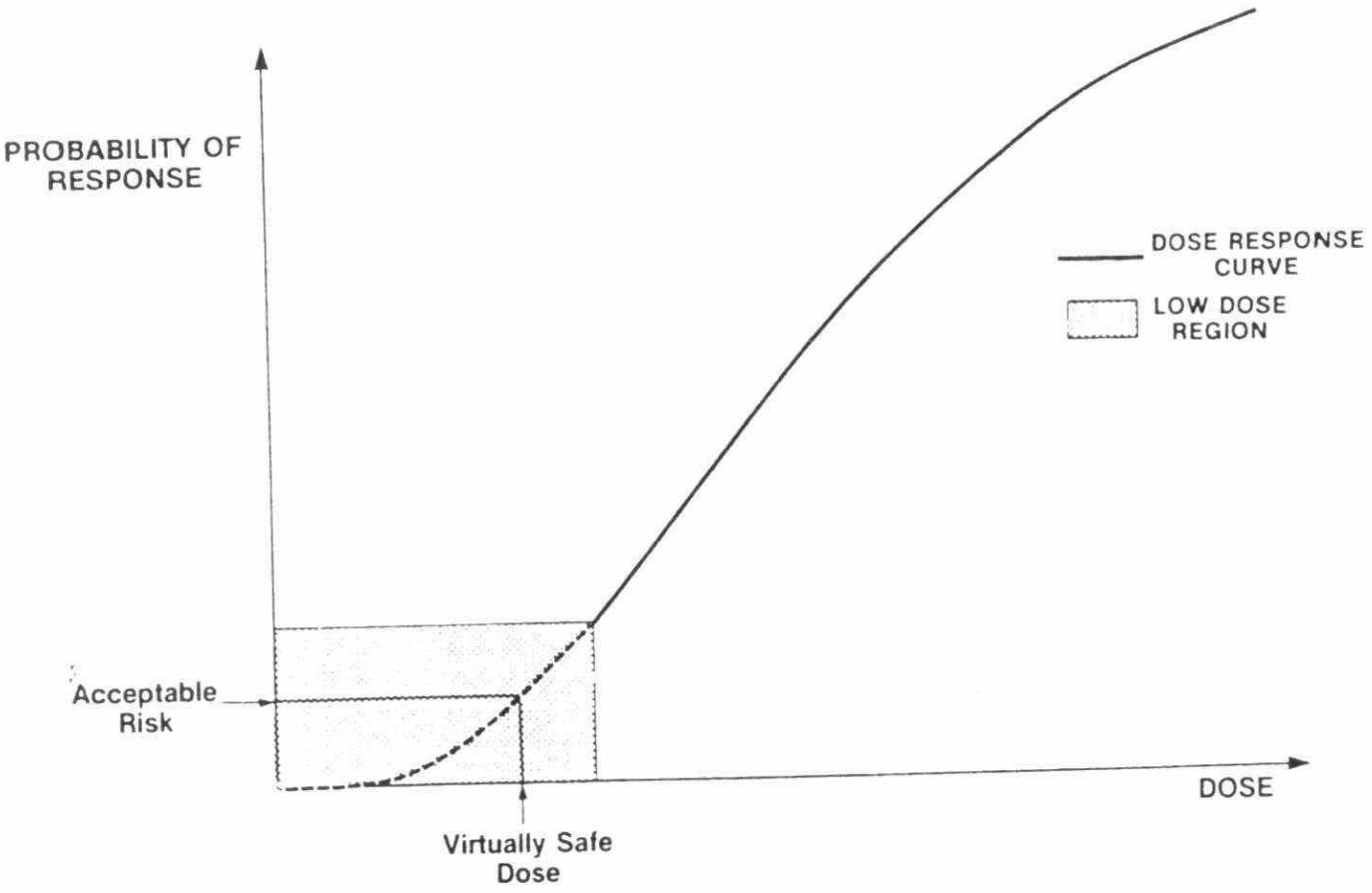

PROBABILITY OF RESPONSE
DOSE RESPONSE CURVE
LOW DOSE REGION
Acceptable Risk
DOSE
Virtually Safe Dose

Figure 1.

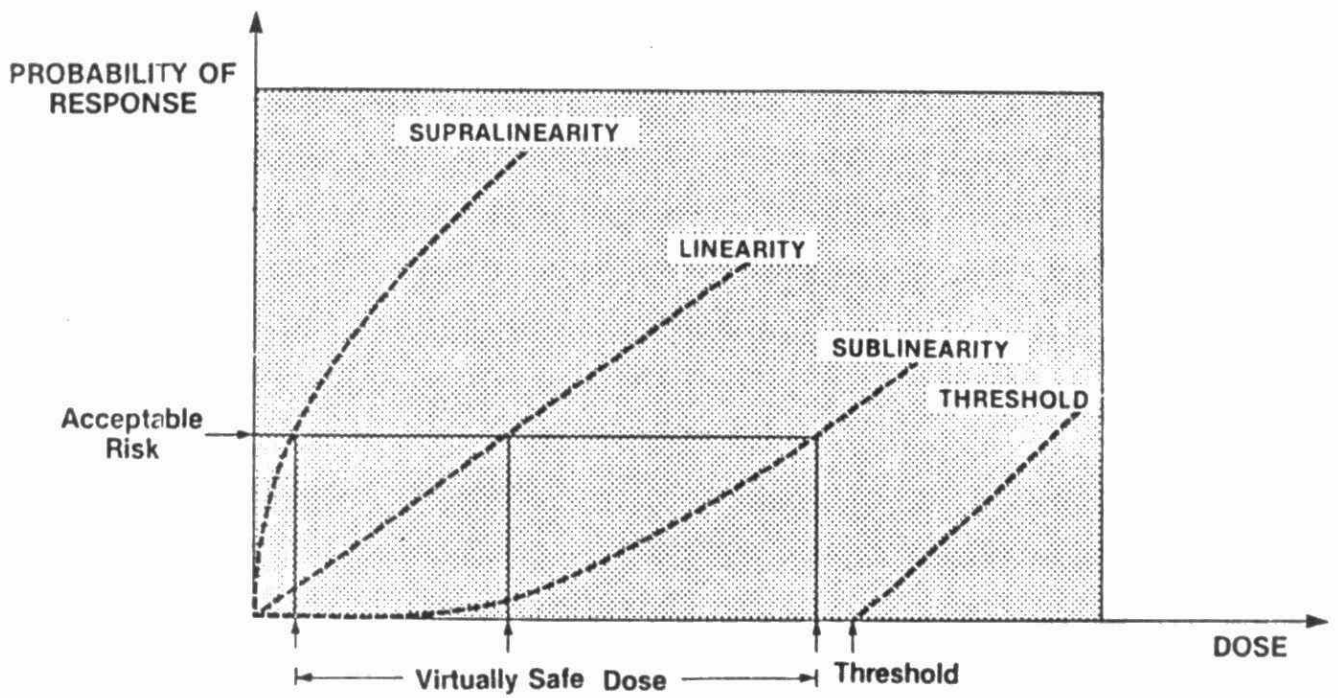
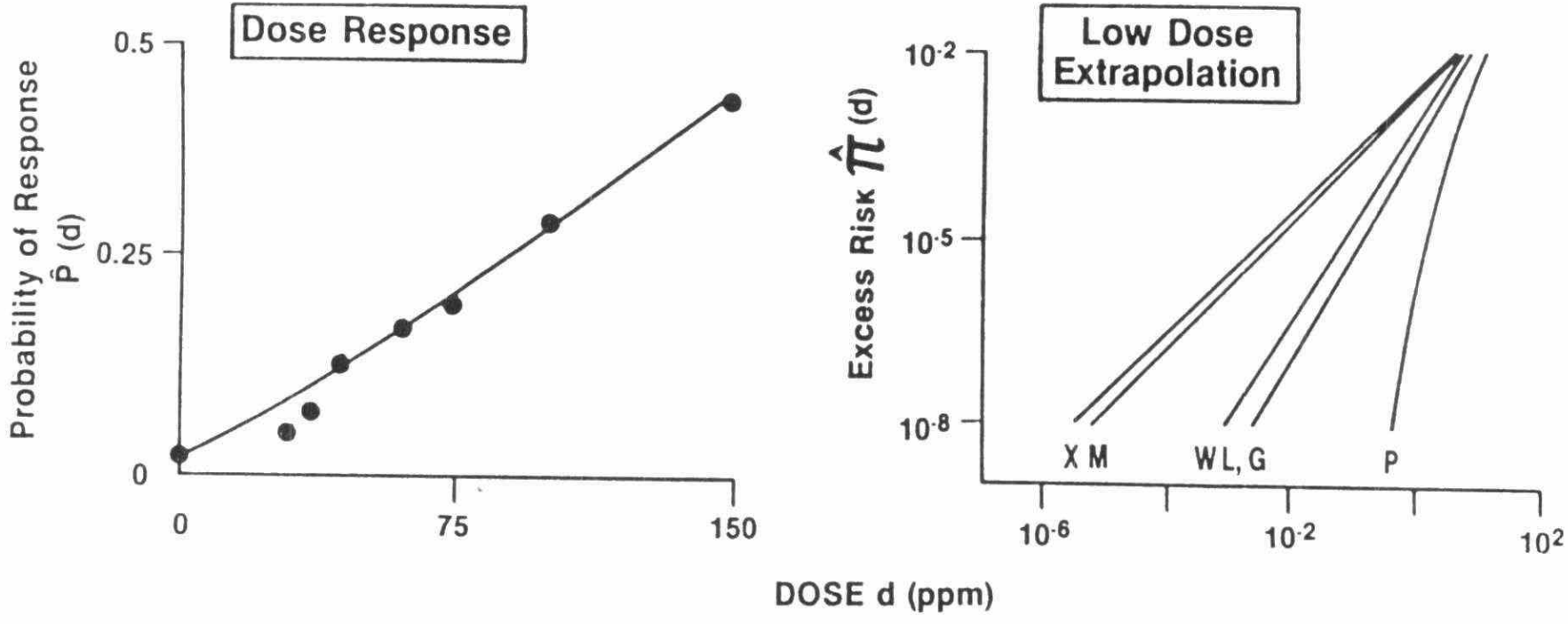

PROBABILITY OF RESPONSE
SUPRALINEARITY
LINEARITY
SUBLINEARITY
THRESHOLD
Acceptable Risk
DOSE
Virtually Safe Dose
Threshold

Figure 2.



X — Linear Extrapolation
M — Multi-Stage Model
W — Weibull Model
L — Logit Model
G — Gamma Multi-Hit Model
P — Probit Model

Figure 3.

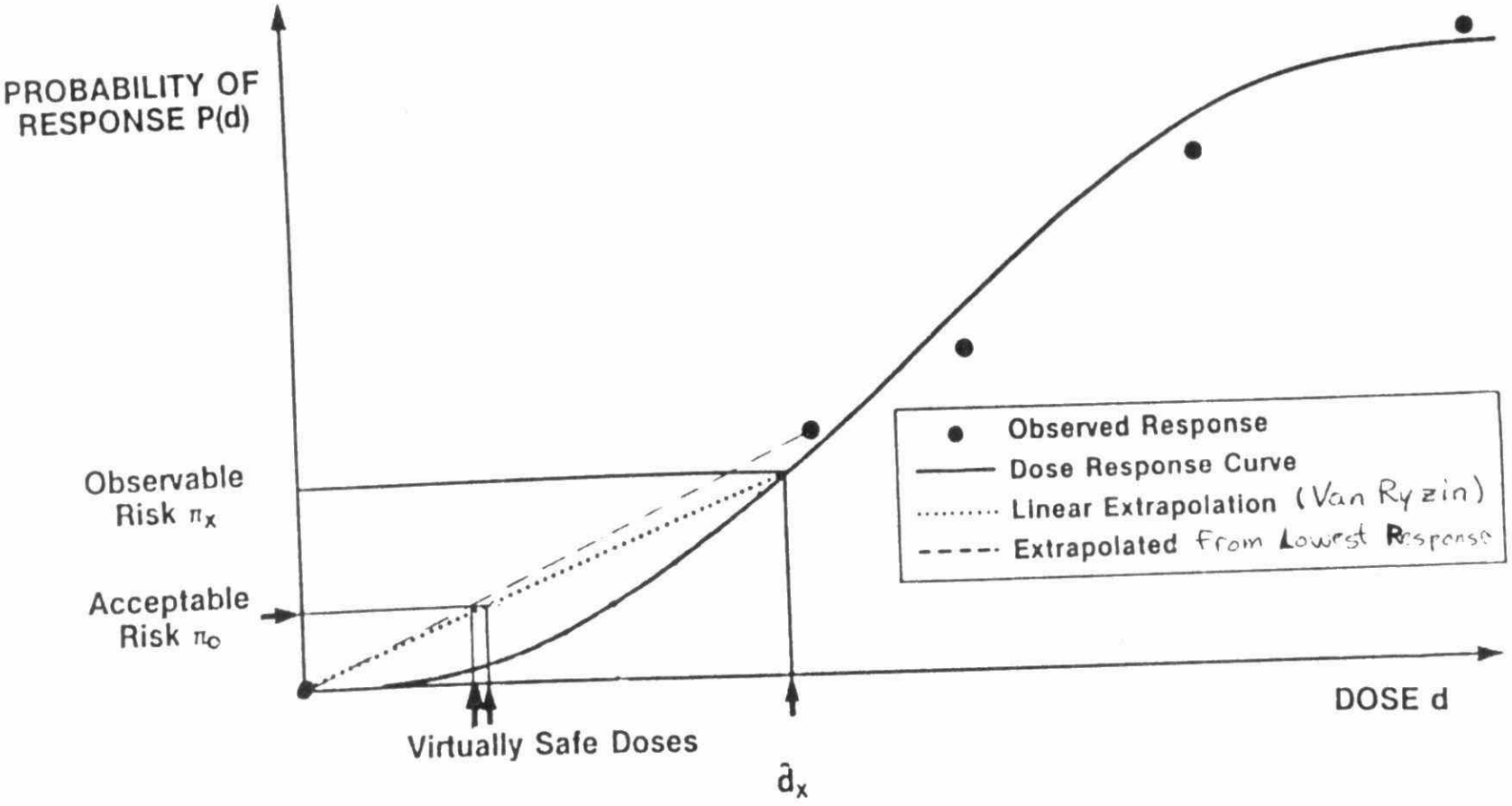

PROBABILITY OF RESPONSE P(d)
Observable Risk π_x
Acceptable Risk π_o
Virtually Safe Doses
d_x
DOSE d
Observed Response
Dose Response Curve
Linear Extrapolation (Van Ryzin)
Extrapolated From Lowest Response

Figure 4.

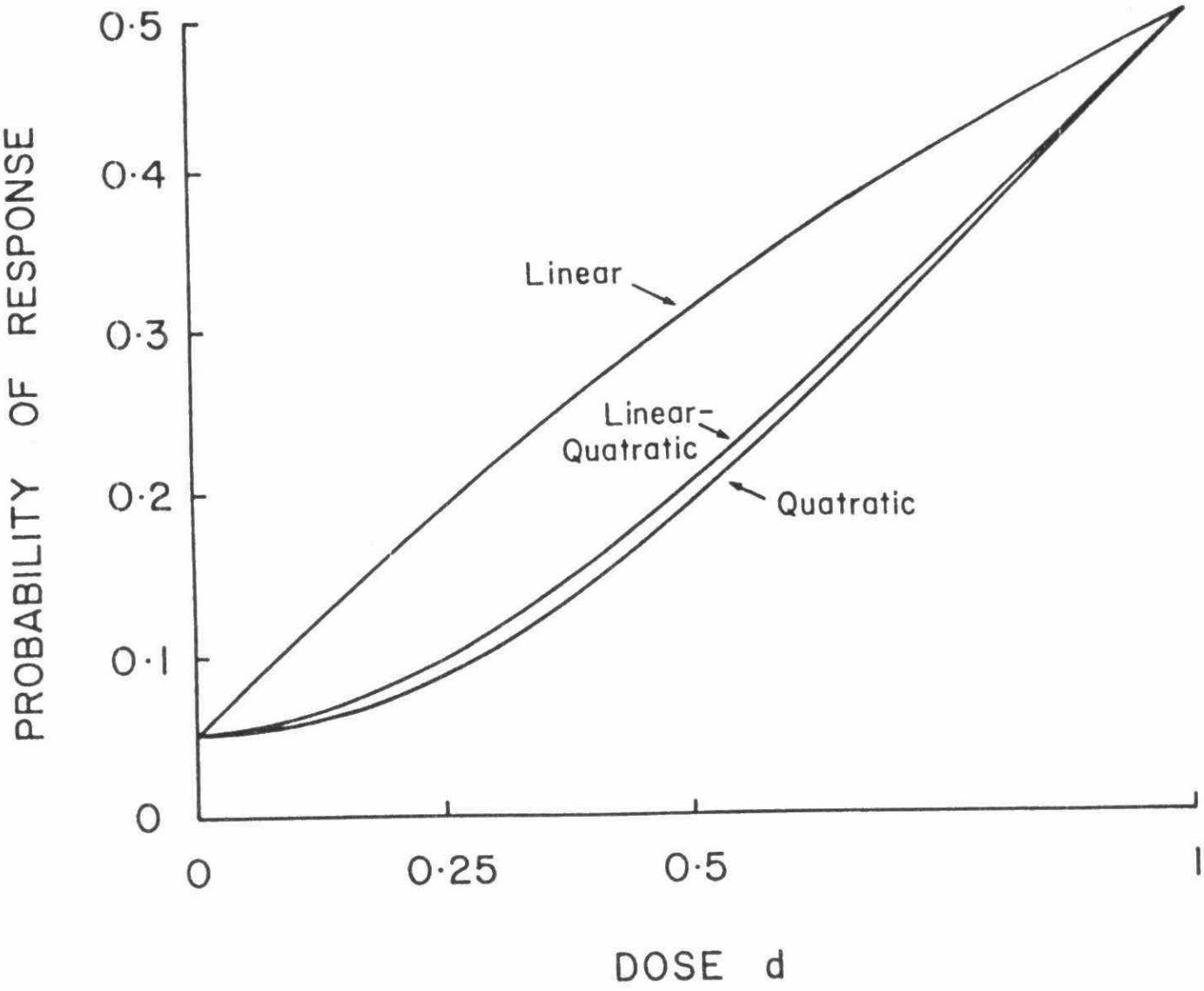

0·5
0·4
0·3
0·2
0·1
0
PROBABILITY OF RESPONSE
Linear
Linear-
Quatratic
Quatratic
0
0·25
0·5
1
DOSE d

Figure 5.

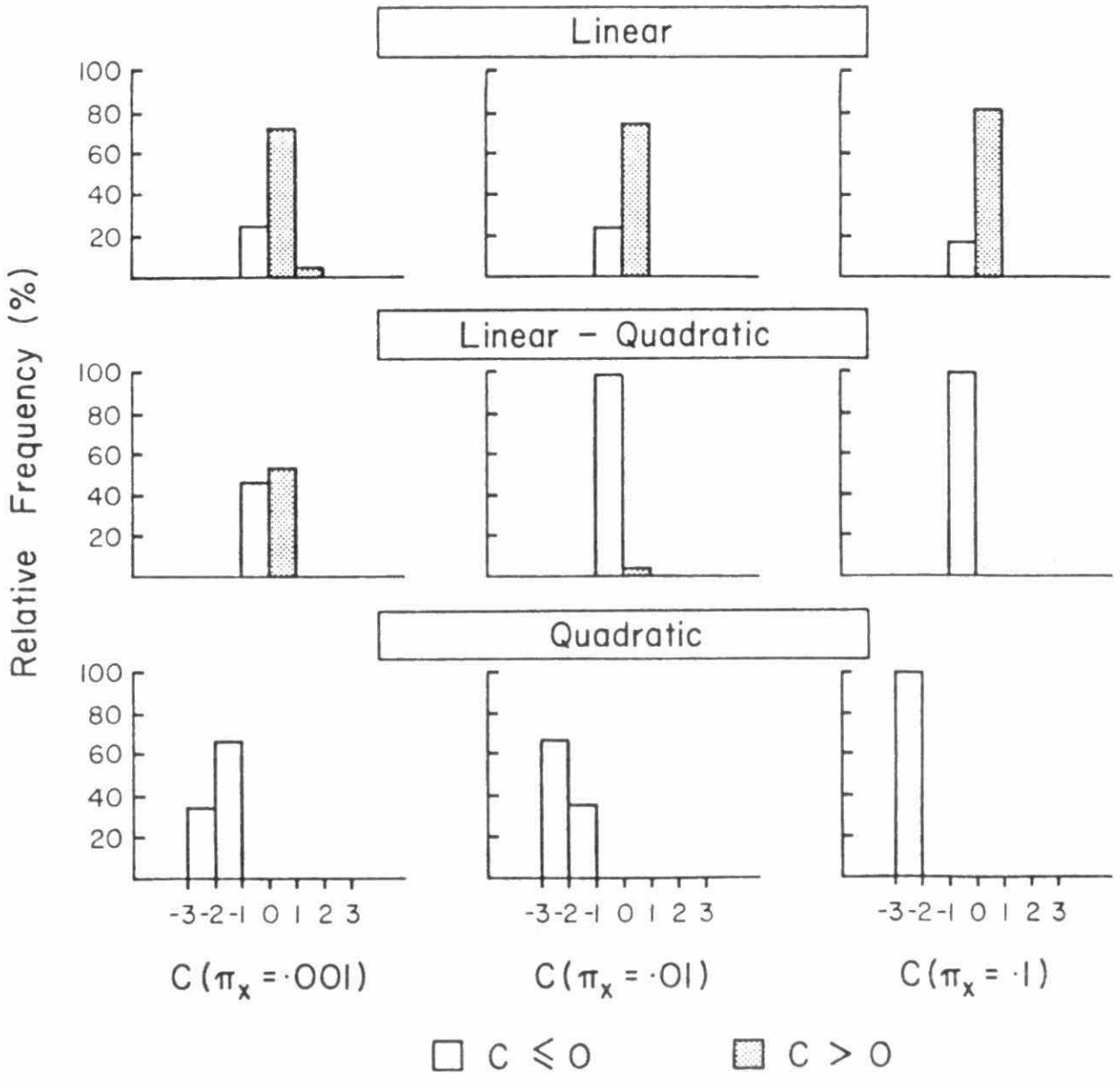

Linear
Linear – Quadratic
Quadratic
Relative Frequency (%)
100
80
60
40
20
-3 -2 -1 0 1 2 3
C(π_x = ·001)
C(π_x = ·01)
C(π_x = ·1)
C ≤ 0
C > 0

Figure 6.

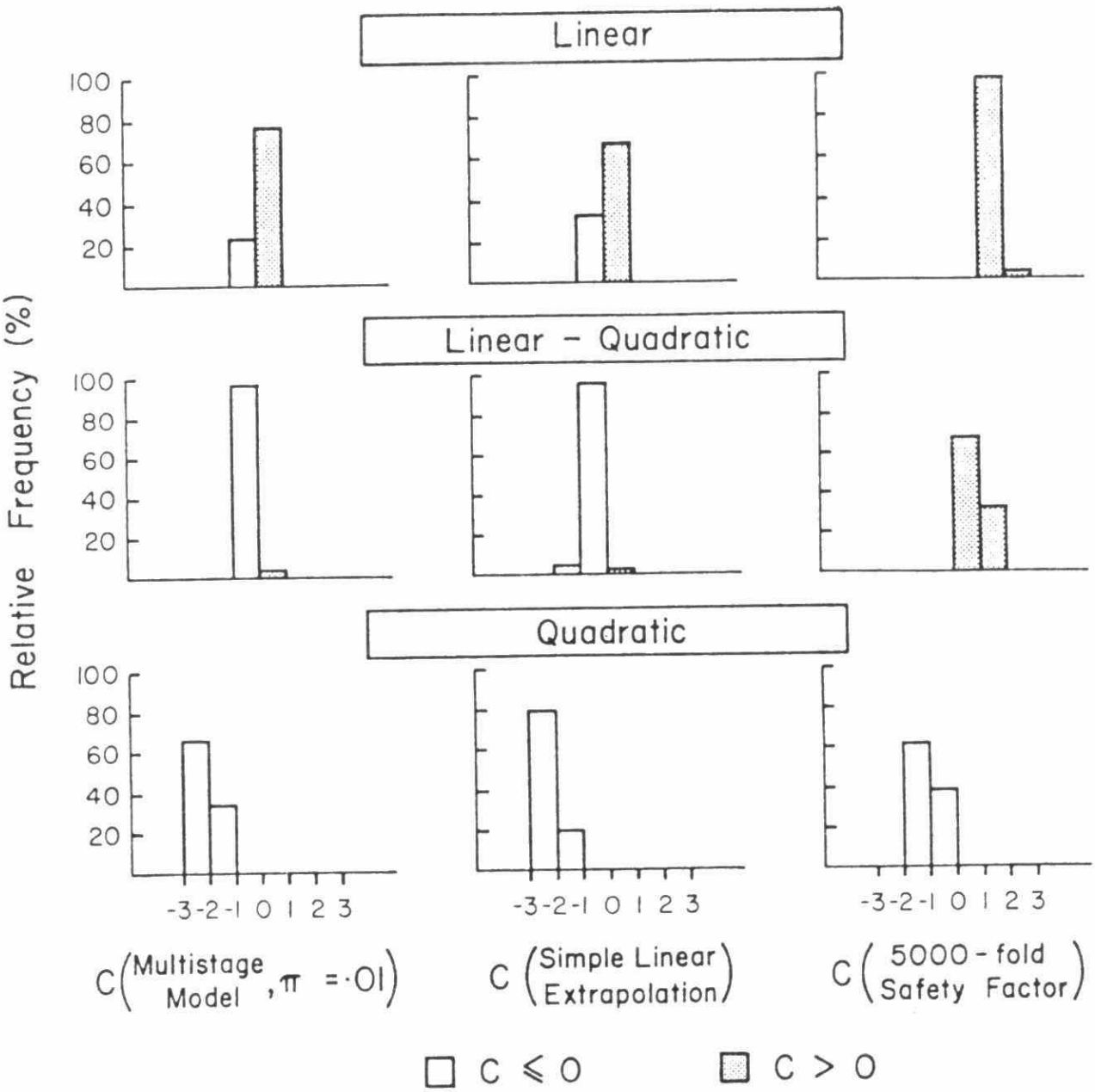

Linear
Linear - Quadratic
Quadratic
Relative Frequency (%)
100
80
60
40
20
-3 -2 -1 0 1 2 3
C(Multistage Model, π = ·01)
C(Simple Linear Extrapolation)
C(5000 - fold Safety Factor)
□ C ≤ 0
▨ C > 0

Figure 7.

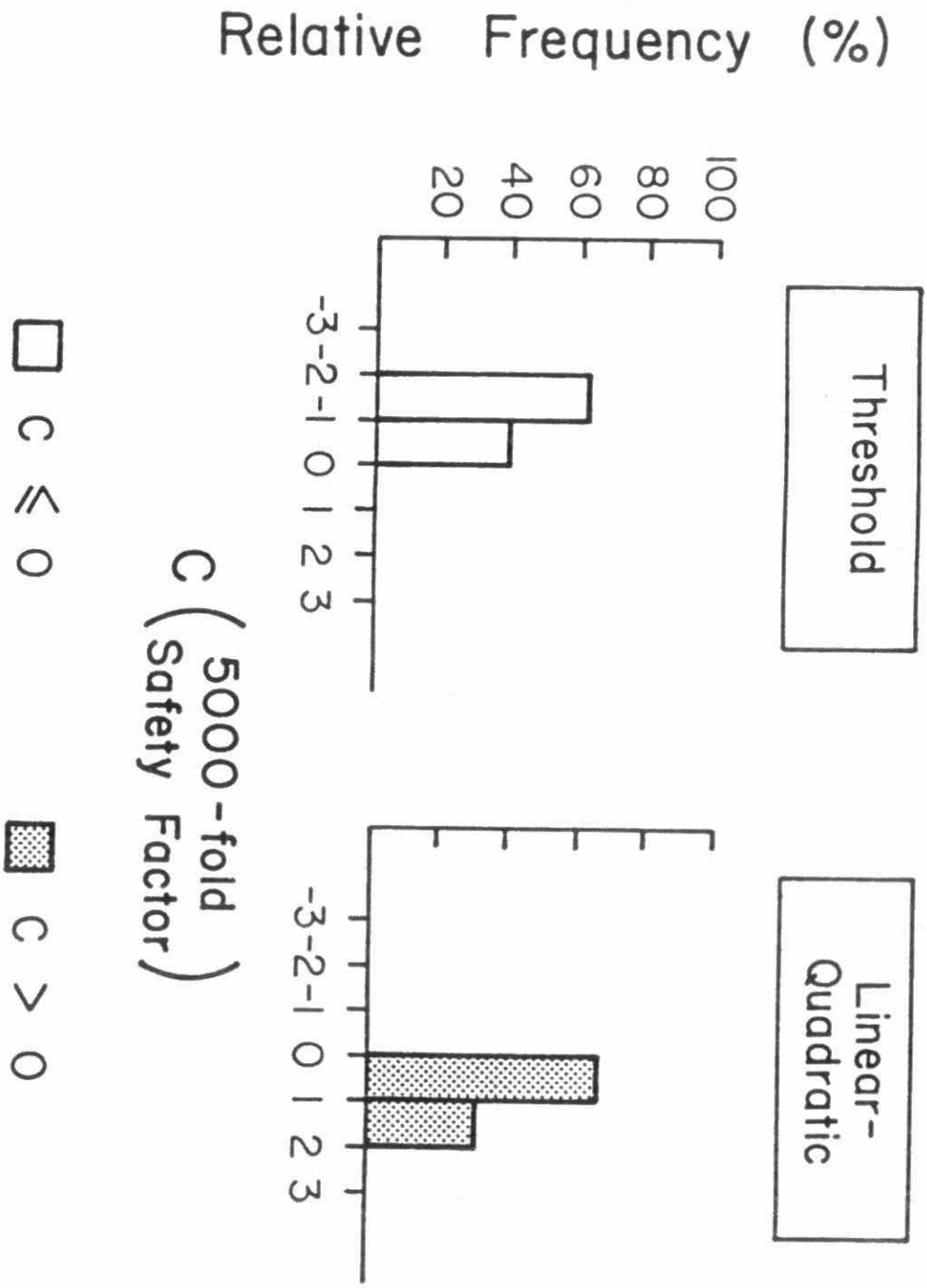

Relative Frequency (%)
100
80
60
40
20
Threshold
Linear-Quadratic
-3 -2 -1 0 1 2 3
C (5000-fold Safety Factor)
C ≤ 0
C > 0

Figure 8.

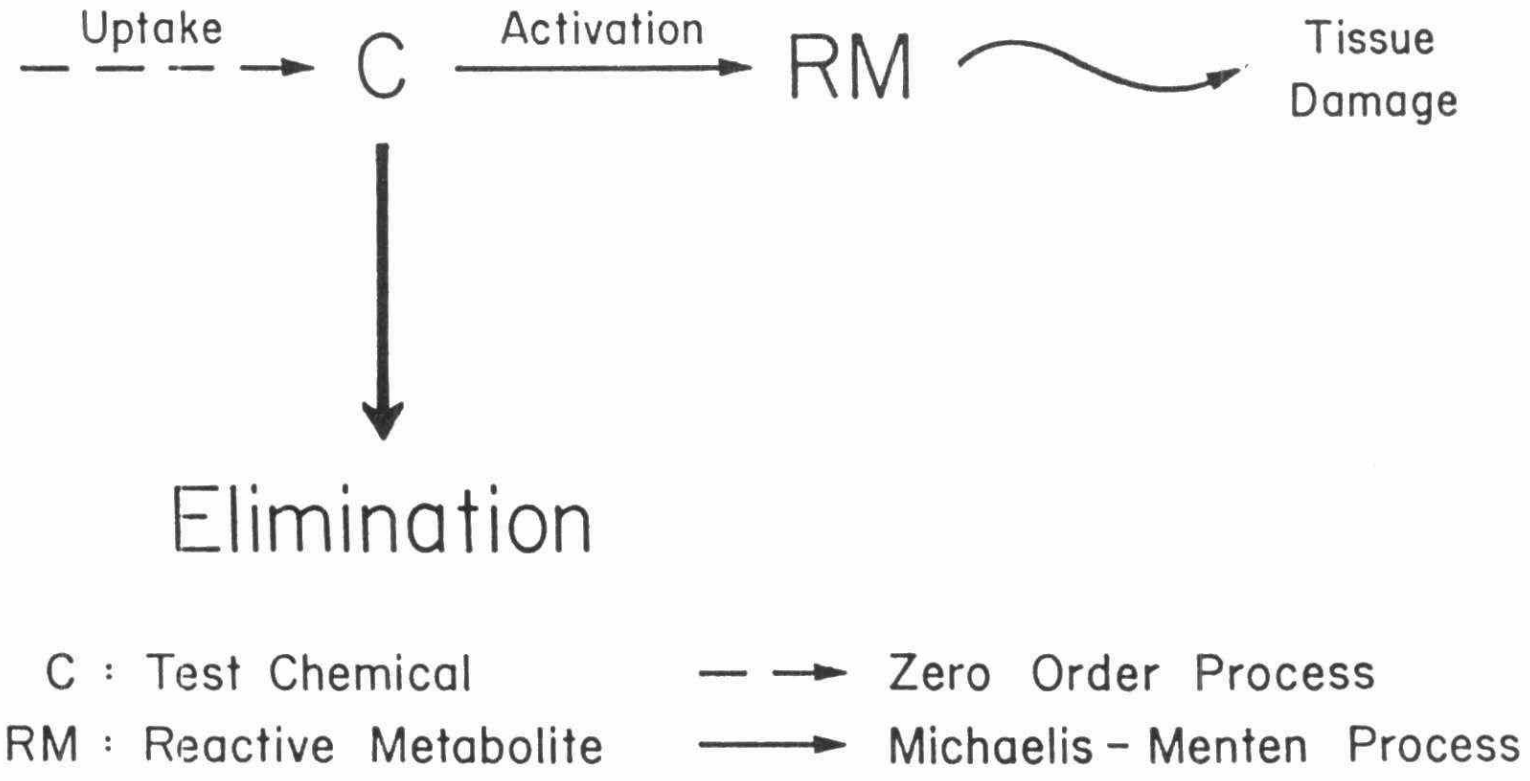

Uptake
C
Activation
RM
Tissue
Damage
Elimination
C : Test Chemical
RM : Reactive Metabolite
Zero Order Process
Michaelis - Menten Process

Figure 9.

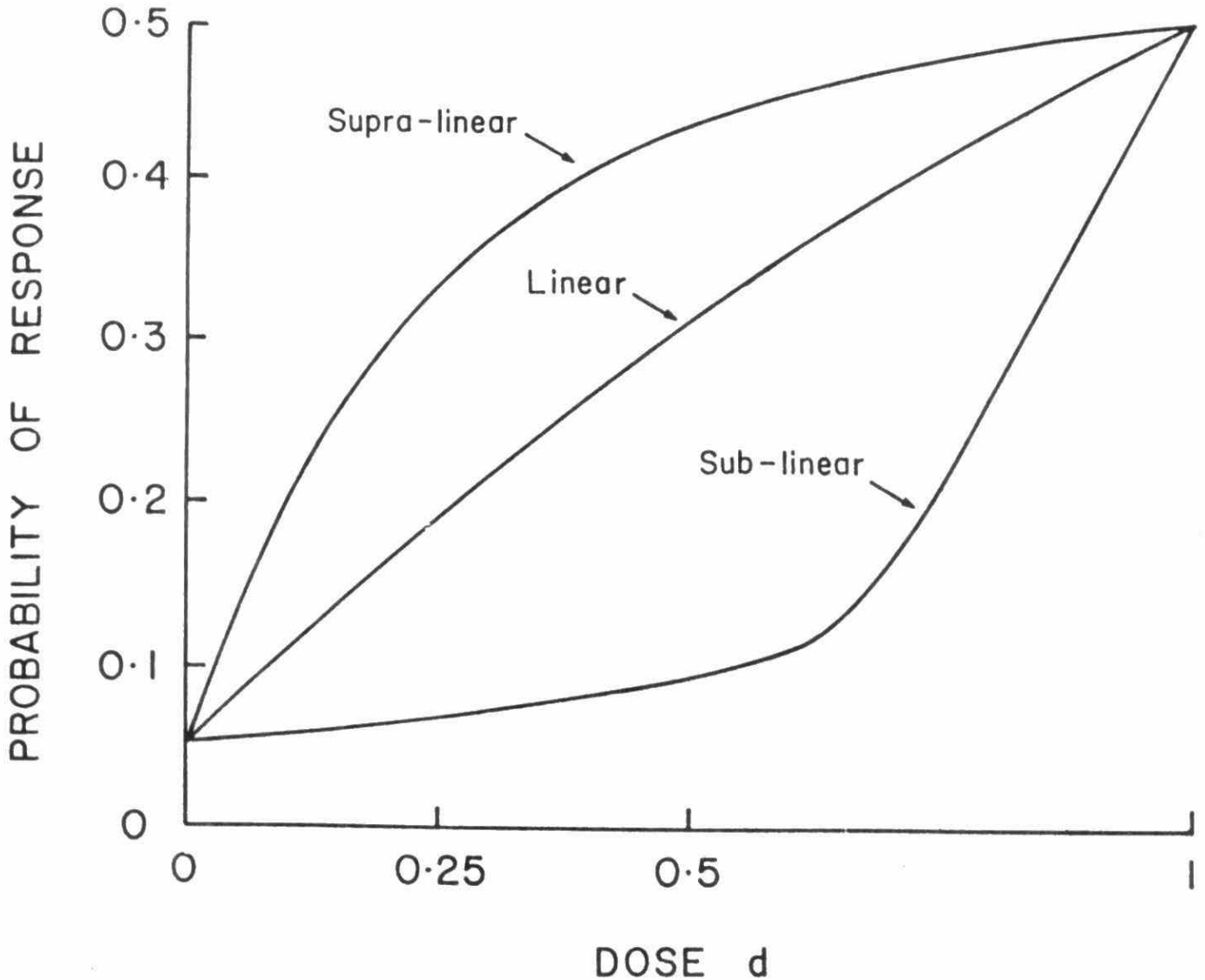

0·5
0·4
0·3
0·2
0·1
0
PROBABILITY OF RESPONSE
Supra-linear
Linear
Sub-linear
0
0·25
0·5
1
DOSE d

Figure 10.

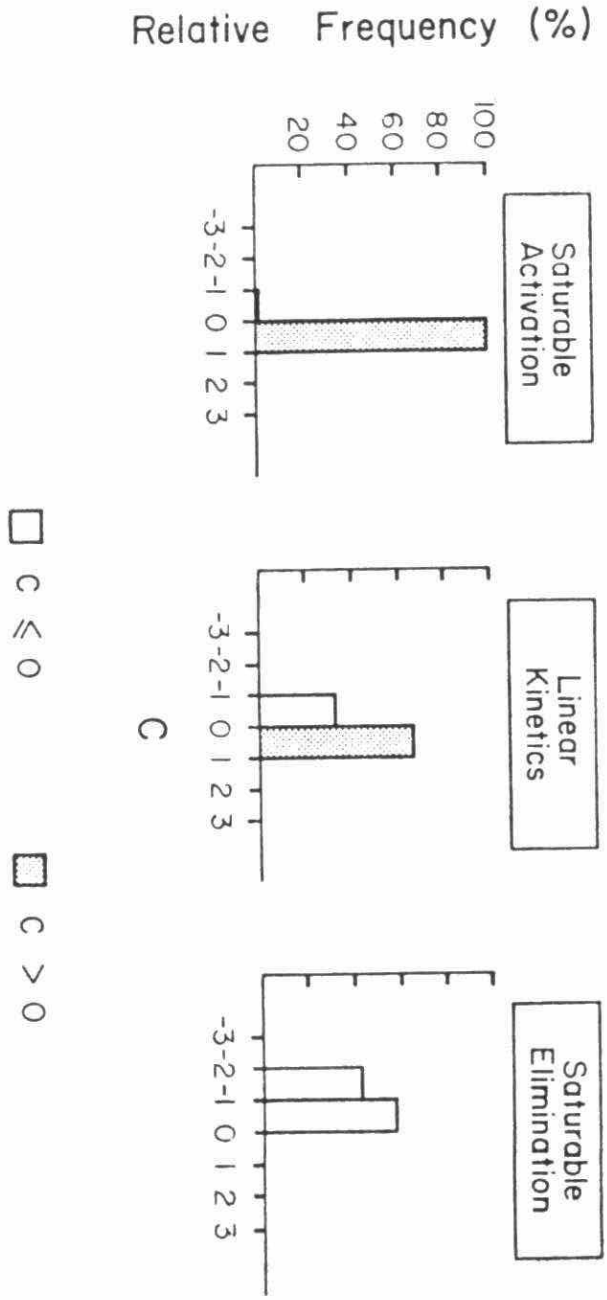

Relative Frequency (%)
100
80
60
40
20
-3-2-1 0 1 2 3
Saturable Activation
Linear Kinetics
Saturable Elimination
C
C ≤ 0
C > 0

Figure 11.

THE STAGES OF CARCINOGENESIS AND THEIR TOXIC EFFECT LEVELS

Douglas Harding, M.D., D.A.B.Path.

One of the great purposes of science is to free society of fear and superstition. An increased understanding of life, biology, physics and all the other components of science has progressively eliminated the fears and superstitions of man to the point that today science is the main foundation of our civilization.

Recently, however, our increasing knowledge concerning chemicals in the environment is paradoxically generating fear in the society and the building up of superstitions such as:

1. There is no safe level of a chemical and even a single molecule can cause death.
2. The only way to protect the environment and the health of living things including man, is by banning the substance.

About ten years ago we in fact knew very little about the chemicals polluting the environment. In order to prudently protect the public health, a list of assumptions were drawn up for administrative or regulatory purposes, and were based not on science, but

on the absence of knowledge. It is these assumptions which has led to the attitude prevalent in society today.

The nine principles put forth by the Environmental Protection Agency (EPA) are as follows:

Table 1

1. A carcinogen is any agent which increases tumor production in man or animals.
2. Well-established criteria exist for distinguishing between benign and malignant tumors; however, even the induction of benign tumors is sufficient to characterize a chemical as a carcinogen.
3. The majority of human cancers are caused by avoidable exposure to carcinogens.
4. While chemicals can be carcinogenic agents only a small percentage are.
5. Carcinogenesis is characterized by its irreversibility and long latency period following the initial exposure to the carcinogenic agent.
6. There is great variation in individual susceptibility to carcinogens.
7. The concept of a threshold exposure level for a carcinogenic agent has no practical significance because there is no valid method of establishing such a level.

8. A carcinogenic agent may be identified through analysis of tumor induction results with laboratory animals exposed to the agent, or on a post hoc basis by properly conducted epidemiological studies.

9. Any substance which produces tumors in animals must be considered a carcinogenic hazard to man if the results were achieved according to the established parameters of a valid carcinogenesis test.

Since the drawing up of these principles science has advanced and where there was lack of knowledge we now have some ideas about the mechanism of carcinogenesis. The pathogenesis of cancer is now thought to occur in stages with the main stages being initiation followed by progression. In many cases, a stage of promotion occurs between initiation and progression.

To understand the change in thinking two definitions are useful. The process as defined by the EPA principles is referred to as a functional definition in which the animal is envisaged as a black box. A chemical is put into the black box and if a neoplasm comes out the chemical is defined as a carcinogen.

The new approach is to develop a mechanistic understanding of the process of tumorogenesis and the

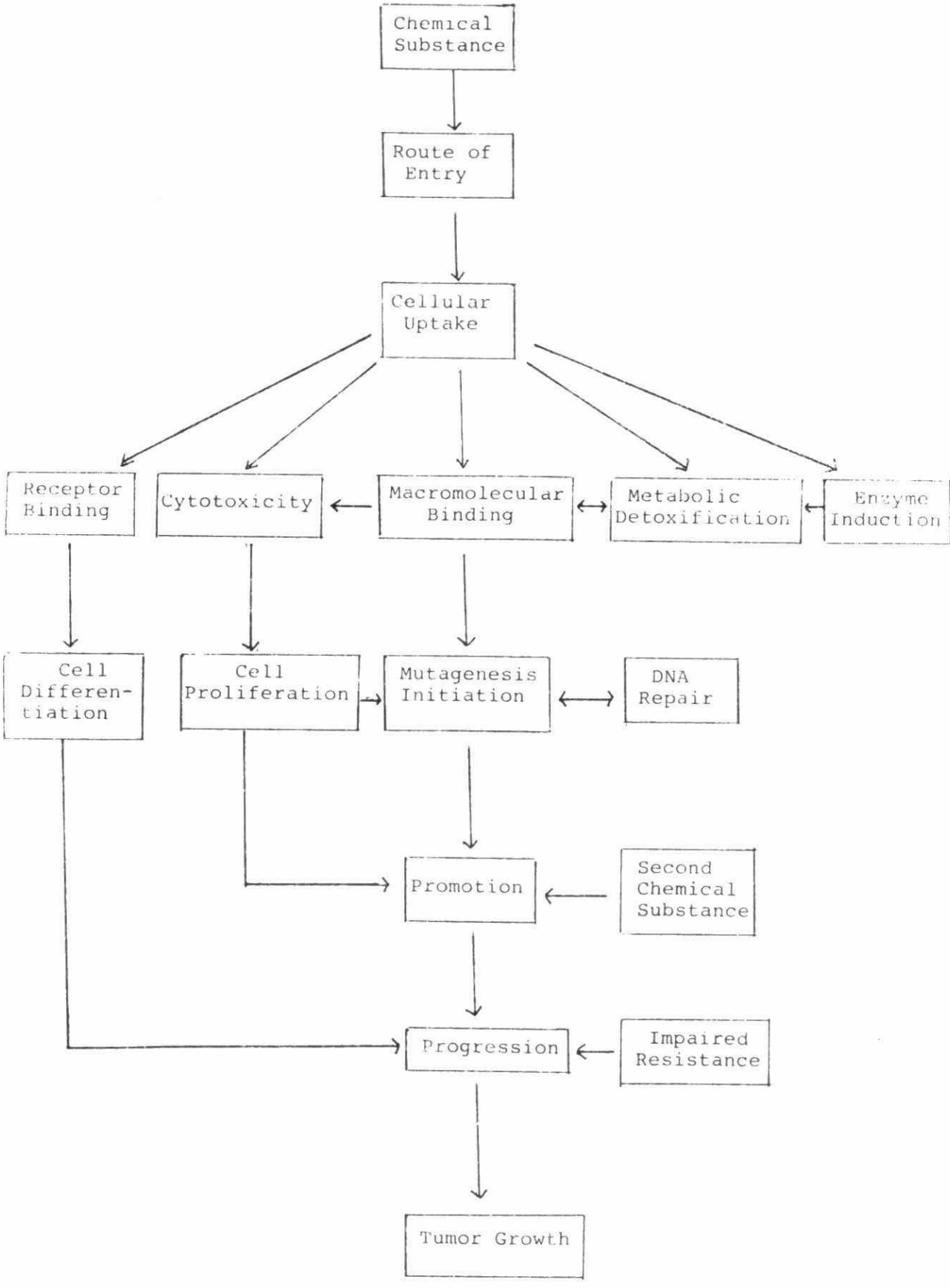
actual chemical reactions undergone by the chemical being tested. The animal as a black box is replaced with an understanding of the chemodynamics of the chemical substance being tested.

The mechanistic approach leads to three processes to be defined which are initiation, epigenetic effects of which promotion is one and cytotoxic effects. It is my belief that epigenetic and cytotoxic effects have thresholds below which there is no untoward consequences upon exposure to the chemical. Initiation on the other hand is not yet understood and a threshold effect level is not definable.

The stages of carcinogenesis is illustrated in the following slides:

Figure 1. Stages of Carcinogenesis

Figure 1. Stages of Carcinogenesis



The mechanistic definitions for the various terms used for the stages of carcinogenesis are as follows:

Table 2 Mechanistic Definitions

A. Genotoxic Substance or Initiators

1. Direct-acting or primary carcinogen.
   Capable of binding directly to DNA and causing a genotoxic reaction.
2. Procarcinogen or secondary carcinogen.
   Requires conversion thru metabolic activation by host or *in vitro* to type 1.
3. Inorganic carcinogen (i.e. a metal)
   Not directly genotoxic but leads to changes in DNA by selective alteration in fidelity of DNA replication.

B. Epigenetic Substances

4. Solid-state carcinogen
   Exact mechanism unknown; usually affects only mesenchymal cells and tissues; physical form vital.
5. Hormone
   Alters endocrine system balance and differentiation; often acts as a promoter.

6. Immunosuppressor

   Mainly stimulates tumor growth by suppression of the immune system.

7. Co-carcinogen

   Not genotoxic or carcinogenic, but enhances the effect of type 1 or type 2 agents when given at the same time.

8. Promoter

   Not genotoxic or carcinogenic, but enhances effect of type 1 or type 2 agents when given subsequently.

We can see now that administering a chemical to an animal which subsequently develops a neoplasm does not indicate that that chemical is a carcinogen.

There are two grades of toxicity now recognized.

(a) A graduated response depending on dose in which the toxic effects become inconsequential at low doses.

(b) A quantum or "all or nothing" effect in which there is no gradation of response. This reaction is applied to initiation or genotoxic effects and is thought to occur at any dose level since no threshold has yet been demonstrated.

The graduated type of toxicity applies to promoters and cytotoxic agents. The gradation of response to a chemical substance is well known and can be illustrated

by the example of puberty. As the male or female sex hormone increases, it will cross the threshold at which sexual development commences. The effect is mediated by chemical receptors in the cells which influence the cells' behavior and cellular proliferation when a given number of the receptors are bound. The binding of chemicals to receptors in the cell is one of the mechanisms by which promotion of initiated cells could occur.

Cytotoxicity is also a gradation response depending on dose. A synergistic response can be seen between cytotoxicity and an initiator such as in the case of formaldehyde. Cell death leads to hyperplasia of surviving cells. The increased rate of mitosis can result in less time for DNA repair to be completed, or in the locking in of mutational events leading to initiation, or could increase the target size for mutational events by the initiator. However, there is a threshold for cytotoxic effects and low doses of the toxic chemical would be inconsequential.

The presence of thresholds is the rule in toxicology and graduated toxicological effects playing a role in carcinogenesis should be separated out. The "all or none" type of reaction is exceptional and was first observed with the carcinogenetic effects of radiation. This observation was then applied to chemicals having mutagenic effects similar to

radiation. Society is best protected from initiators by continuing to use risk analysis and open public discussion to settle the acceptable risk question.

The use of risk analysis no longer appears necessary for substances not having "all-or-none" toxicity. Well conducted toxicological research should be able to demonstrate the dose response gradient and the approximate threshold. Uncertainty factors could be applied to the reliable no observed effect level (NOEL) or to the dose causing an effect in 1% of the animal (ED01) to drive at a safe dose or concentration.

If the environment is full of promoters as many people believe, then the addition of any initiating carcinogen would result in an epidemic of cancer. This has not happened and is a common sense understanding that the sea of promoters around us must be present at levels below a threshold for no such epidemic is occurring. It has been pointed out many times that the rate of cancer has not increased over many years except for lung cancer almost all of which can be ascribed to cigarette smoking.

Summary

The generalized definitions given by the E.P.A. should be abolished and a mechanistic set of definition based on the stages of carcinogenesis substituted.

Experiments should be designed to determine the

biochemical mechanism involved and to eliminate cytotoxic interference. No generalized statement that a chemical is a carcinogen can be made simply because it causes tumors to develop in animals or because humans exposed to the chemical at work are dying of cancer.

Risk analysis should be applied only to genotoxic substances having an "all or none" toxic response. It is not sensible to apply risk analysis to epigenetic or non-genotoxic substances having a graduated toxic response. For this group of chemicals, an experimentally well defined NOEL or ED01 can be used as an endpoint. A safety or uncertainty factor can then be applied to drive at a level below the toxic threshold level of the population.

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